ANALYSIS

By

DR.VARSHA R. DESHPANDE

Dissertation submitted to the

B.L.D.E. University, Vijayapura, Karnataka.

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

DR. R.M.POTEKAR M.D.

PROFESSOR,

DEPARTMENT OF PATHOLOGY

B.L.D.E. UNIVERSITY'S, SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA.

2018

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"PANCYTOPENIA- A CLINICO HEMATOLOGICAL ANALYSIS"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. M. POTEKAR**, Professor, Department of Pathology, and co- guide **Dr. L. S. PATIL,** Professor, Department of General Medicine B.L.D.E.U's Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapura, Karnataka.

Date: **Dr. VARSHA R. DESHPANDE**

Place: Vijayapura

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"PANCYTOPENIA- A CLINICO HEMATOLOGICAL ANALYSIS"** is a bonafide research work done by **Dr. VARSHA R. DESHPANDE** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapura

Dr. R. M. POTEKAR

Professor
Department of Pathology,
B.L.D.E.U's Shri B. M. Patil
Medical College, Hospital &
Research Centre, Vijayapura,
Karnataka

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"PANCYTOPENIA- A CLINICO HEMATOLOGICAL ANALYSIS"** is a bonafide research work done by **Dr. VARSHA R. DESHPANDE** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapura

Dr. L. S. Patil

Professor
Department of General Medicine,
B.L.D.E.U's Shri B. M. Patil
Medical College, Hospital &
Research Centre, Vijayapura,
Karnataka

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

ENDORSEMENT BY HEAD OF DEPARTMENT

This is to certify that the dissertation entitled **"PANCYTOPENIA- A CLINICO HEMATOLOGICAL ANALYSIS"** is a bonafide research work done by **Dr. VARSHA R. DESHPANDE** under the guidance of, **Dr. R. M. POTEKAR** M.D. Professor, Department of Pathology, and co- guide **Dr. L. S. PATIL,** Professor, Department of General Medicine, Shri B. M. Patil Medical College, Vijayapura in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapura

Dr. B. R. YELIKAR

Professor and H.O.D,
Department of Pathology,
B.L.D.E.U's Shri B. M. Patil
Medical College, Hospital &
Research Centre, Vijayapura,
Karnataka.

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

ENDORSEMENT BY PRINCIPAL /

HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"PANCYTOPENIA- A CLINICO HEMATOLOGICAL ANALYSIS"** is a bonafide research work done by **Dr. VARSHA R. DESHPANDE** under the guidance of, **Dr. R. M. POTEKAR** M.D. Professor, Department of Pathology, and co- guide **Dr. L. S. PATIL,** Professor, Department of General Medicine, Shri B. M. Patil Medical College, Vijayapura, in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapura

Dr. S. P. GUGGARIGOUDAR

Principal,
B.L.D.E.U's Shri B.M. Patil
Medical College, Hospital &
Research Centre, Vijayapura,
Karnataka.

B.L.D.E. UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPURA

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the B.L.D.E. University, Karnataka shall have the rights to preserve, use and disseminate this dissertation / thesis in print or electronic format for academic / research purpose.

Date: **Dr. VARSHA R. DESHPANDE**

Place: Vijayapura

ACKNOWLEDGEMENT

This study could not have been accomplished without the grace of God. I would like to express my sincere gratitude to a number of people, who have supported, guided and encouraged me throughout the duration of my project.

I remain indebted to my esteemed and honorable teacher, **Dr. R. M. POTEKAR,** Professor, Department of Pathology, for his dedication and providing invaluable guidance, precise approach, constructive criticism, unremitting encouragement and meticulous supervision to constantly improve upon my study.

I acknowledge my gratitude to my co- guide **Dr. L. S. PATIL,** Professor, Department of General Medicine, for his continuous support and encouragement.

I would like to express my deepest gratitude to **Dr. B.R.YELIKAR** Professor and H.O.D, Department of Pathology, for his patience, motivation, enthusiasm and immense knowledge. He has had a profound influence on both my personal growth and professional pursuits.

I am privileged to have such approachable and supportive staff. I am thankful to **Dr. S. U. Arakeri,** Professor, **Dr. S. B. Hippargi**, Professor, **Dr. M. H. Karigoudar** Professor, **Dr. Girija Patil** Associate Professor, **Dr. Prakash M. Patil** Associate Professor, **Dr. Vijayalaxmi S. Patil** Assistant Professor, **Dr. Anita P. Javalgi** Assistant Professor, **Dr. Savitri M. Nerune,** Assistant Professor, **Dr. Mamatha K.** Assistant Professor and **Dr. Sneha Jawalkar,** Assistant Professor, for their advice, supervision, concern and feedback during this period.

I am also thankful to all my batchmates, seniors and juniors who have helped and encouraged me during my work.

I am very grateful to all the non teaching staffs of Department of Pathology, who have helped me during this work.

I am also thankful to Statistician **Dr.Vijaya Sorganvi** and Librarian **Mr. Harsha Maga**, who have helped me during my study.

I am indebted and grateful to my father, **Mr. Vishwanath R. Hosur**, for making me what I am today and instilling in me his virtues, and my mother, **Mrs. Pushpa,** for her incessant prayers and blessings.

No words are enough to express the gratitude I feel for the constant support extended by my father in law **Prof. Ravindra G. Deshpande** and my mother in law, **Mrs. Rekha R. Deshpande** during this entire course of study. Their encouragement and moral support have made this period much less intense.

My professional growth has been nurtured by my understanding husband **Dr. Ramesh R. Deshpande,** who has been a constant source of inspiration and encouragement during my study. I am immensely grateful to him, for having staunch faith in me and my capabilities.

My children, **Akanksha** and **Akshaj,** were most important source of inspiration during this study.

Last but not the least, my sincere gratitude to all my study subjects for their cooperation to this study.

Date:

Place: Vijayapura **DR. VARSHA R. DESHPANDE**

LIST OF ABBREVATIONS USED

HSC	Hematopoietic Stem Cell
CFU	Colony Forming Units
GM- CSF	Granulocyte- Macrophage Colony Stimulating Factor
G- CSF	Granulocyte -Colony Stimulating Factor
NK	Natural Killer
RBC	Red Blood Cells
WBC	White Blood cells
WHO	World Health Organization
Hb	Hemoglobin
Hct	Hematocrit
SD	Standard Deviation
AA	Aplastic Anemia
EBV	Ebstein Barr Virus
PNH	Paroxysmal Nocturnal Hemoglobinuria
MDS	Myelodysplastic Syndrome
AML	Acute Myeloid Leukemia
ALL	Acute Lymphoblastic Leukemia
FA	Fanconi Anemia
hTERC	human Telomerase RNA Component
CAMT	Congenital Amegakaryocytic Thrombocytopenia
TPO	Thrombopoeitin

SDS	Shwachman- Diamond Syndrome
PMF	Primary Myelofibrosis
MF	Myelofibrosis
CML	Chronic Myeloid Leukemia
PV	Polycythemia Vera
ET	Essential Thrombosis
LDH	Lactate Dehydrogenase
PNH	Paroxysmal Nocturnal Hemoglobinuria
GPI	Glycosylphoshpatidylinositol
MM	Multiple Myeloma
SLE	Systemic Lupus Erythematosis
HPS	Heamophagocytic Syndrome
HIV	Human Immunodeficiency Virus
HPL	Hemophagocytic Lymphohisticytosis
EDTA	Ethylene Diamine Tetra Acetic Acid.
TLC	Total Leucocyte Count
MCHC	Microcytic Hypochromic
NCNC	Normocytic Normochromic
NCHC	Normocytic Hypochromic
MA	Megaloblastic Anemia
CDA	Combined Deficiency Anemia
IDA	Iron Deficiency Anemia
AL	Acute Leukemia
HS	Hypersplenism

NHL	Non Hodgkin Lymphoma
PS	Peripheral Smear
BMA	Bone Marrow Aspiration
BMB	Bone Marrow Biopsy
MPO	Myeloperoxidase
APML	Acute Promyelocytic Leukemia

ABSTRACT

BACKGROUND:

Pancytopenia is a common hematological finding characterized by the presence of anemia, leucopenia and thrombocytopenia. It is a triad of findings which result from a number of disorders affecting the bone marrow primarily or secondarily. Pancytopenia has multiple causes, ranging from simple drug induced marrow hypoplasia, nutritional deficiency (megaloblastic anemia) to life threatening illnesses like fatal aplastic anemia and leukemias. The underlying etiologies vary in different groups of population depending on their nutritional status, age groups, environmental conditions, prevalence of different infections and genetic mutations.

The presenting complaints are mainly due to anemia, leucopenia and thrombocytopenia. The underlying pathology and severity of pancytopenia determine the management and prognosis of these patients. Thus, identification of underlying etiology will help in appropriate management of cases of pancytopenia.

OBJECTIVE:

The objective of this study was to evaluate patients with pancytopenia and analyze the clinical profile, hematological parameters, peripheral smear findings, bone marrow morphology and etiological spectrum.

MATERIALS AND METHODS:

A cross sectional, hospital based study was conducted in the Department of Pathology at BLDEU'S Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapur from 1st November 2015 to 30th June 2017. Total 120 cases of pancytopenia of all age group were studied to analyze clinical presentations,

peripheral smear findings and bone marrow morphology. The underlying various etiologies were assessed along with other relevant investigations done on the respective patients.

RESULTS:

Among 120 cases studied, the age of patients ranged from 1 to 85 years, with male preponderance. The most common presenting complaint was generalized weakness followed by fever. On physical examination pallor was the most common finding followed by splenomegaly and hepatomegaly. Bone marrow was hypercellular in 85% of cases and hypocellular in 4.17% of cases. The commonest cause of Pancytopenia was Megaloblastic anemia (65%), followed by Combined deficiency anemia (13.33%), Iron deficiency anemia (10.83%), Acute leukemia (3.33%), Aplastic anemia (3.33%), Alcoholic liver disease (1.7%), Hypersplenism (0.83%), Malaria (0.83%) and Myelodysplastic syndrome (0.83%).

CONCLUSION:

The diagnostic clues obtained from evaluation of heamogram and bone marrow study were useful in the early diagnosis and helpful in planning further investigations and management of pancytopenic patients.

Megaloblastic anemia, Combined deficiency anemia and Iron deficiency anemia are the common cause of pancytopenia, suggesting a high prevalence of nutritional deficiency in this part of India. Therefore it is recommended that necessary steps be taken to correct such nutritional deficiencies.

KEY WORDS: Pancytopenia, Megaloblastic anemia, Combined deficiency anemia

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INTRODUCTION

Pancytopenia is a common clinico- hematological entity encountered in our routine clinical practice. [1]

Pancytopenia is the simultaneous presence of anemia, leucopenia and thrombocytopenia i.e. when the hemoglobin is less than 13.5g/dl in males or 11.5g/dl in females, the leucocyte count is less than 4000 cells /µl and the platelet count less than 150,000/µl . [2, 3]

Peripheral pancytopenia is a manifestation of disorders of bone marrow, either primary or secondary. It develops from various mechanisms such as suppression of normal marrow growth and differentiation or destruction of marrow by toxins, resulting in decrease in hematopoietic cell production. Other mechanisms include ineffective erythropoiesis with premature death of cells in the marrow, removal of defective cells in the circulation, antibody mediated destruction of cells and sequestration of normal blood cells in hypertrophied and overactive reticuloendothelial system.[4, 5]

The etiology of pancytopenia varies among different population groups depending on their age, nutritional status, environmental conditions and prevalence of infection in different areas. [6]

The underlying hematogical diseases vary with geographical distribution and genetic inheritance. [7, 8,]

The presenting symptoms of pancytopenia are usually attributable to anemia, leucopenia and thrombocytopenia. The usual symptoms presented by the patient due

to anemia are fatigue and weakness, due to leucopenia there is increased susceptibility to infections and excessive bleeding due to thrombocytopenia. [5]

Pancytopenia often presents a diagnostic challenge, because of wide range of potential etiologies including nutritional deficiencies, infections, neoplastic entities and bone marrow failure syndromes. [9]

The complete hematological work up including a good peripheral blood smear examination, bone marrow aspiration and biopsy with clinical correlation is of utmost importance to evaluate the cause of pancytopenia and planning further investigations and treatment. [10]

Bone marrow aspirate is useful to study the cytological details and biopsy allows the study of cellularity of marrow, detection of focal lesions and extent of infiltration by various pathological entities. [3]

Underlying etiopathogenesis and severity of pancytopenia determine the treatment modality and prognosis of the patients. Therefore identifying the correct etiopathology helps in the appropriate diagnostic and therapeutic approach. [8]

The present study has been undertaken to evaluate the etiology, clinical profile, peripheral smear and bone marrow morphology of patients with pancytopenia. There by, this study would help in planning the appropriate diagnostic and therapeutic approach in patients with pancytopenia.

OBJECTIVE OF THE STUDY

The objective of this study is to evaluate patients with pancytopenia and analyze the:

- Clinical profile
- Hematological parameters
- Peripheral smear findings
- Bone marrow morphology and
- Etiological spectrum

HEMATOPOIESIS:

Bone marrow provides a unique microenvironment for the orderly proliferation, differentiation and release of blood cells. [11]

During the course of development from embryonic to adult life, formation of blood cells occurs at different anatomical sites. It begins in the yolk sac of the embryo, and then shifts to the liver and to a lesser extent to the spleen. Bone marrow starts taking over the function of hematopoiesis from the fourth month of fetal life and serves as the only important source of blood cell production after birth. [12] These blood cells show great structural and functional differences. Despite of these differences, majority of the blood cells arise from a single hematopoietic stem cell (HSC). The processes of production of different blood cells from HSCs are collectively known as *hematopoiesis*. [13]

These HSCs have two distinct and important properties which are required for the continuous maintenance of hematopoiesis viz., pluripotency and self renewal capacity. Pluripotency is the ability of single HSC to produce all mature forms of blood cells. Self-renewal is the capacity of one of the daughter cells from HSC to renew to prevent stem cell depletion. [11]

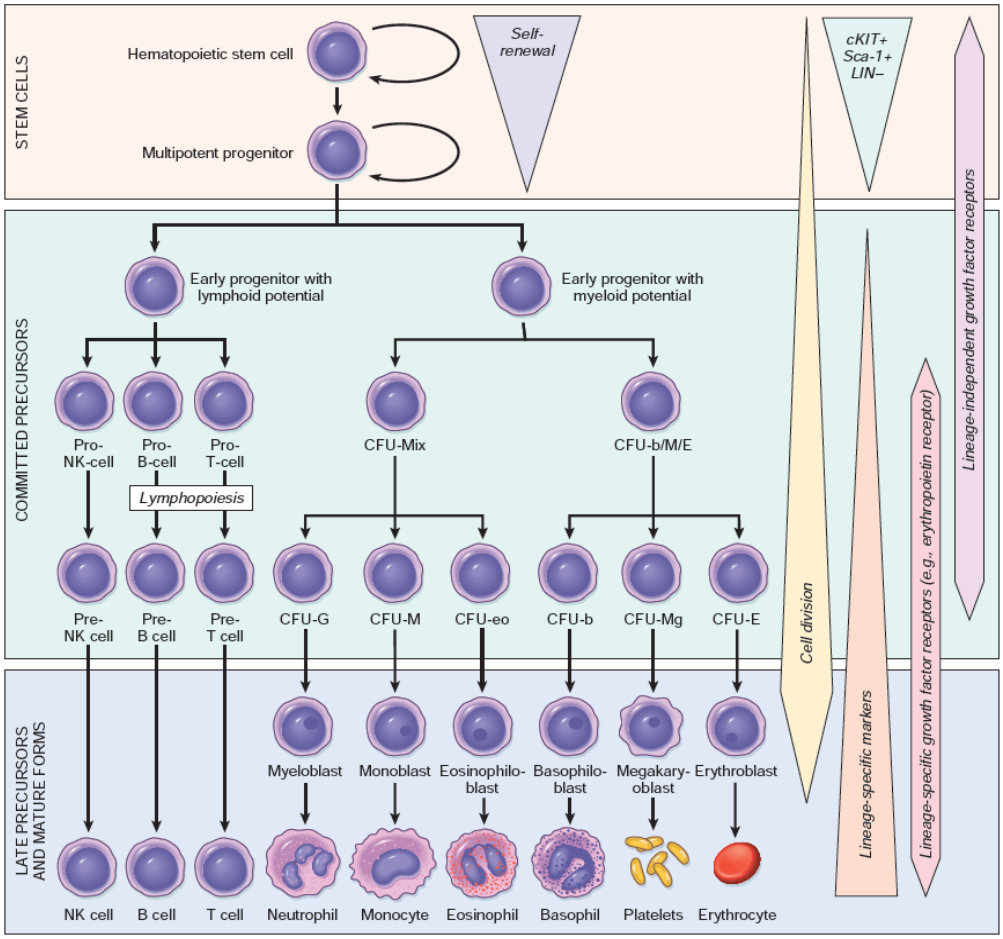
HSCs produce several kinds of early progenitor cells which have restricted differentiation capacity. These cells differentiate ultimately to produce lymphoid and myeloid cells. Early progenitor cells gives rise to colony forming units (CFUs), which have differentiation towards specific lineage. These CFUs produce group of cells composed of specific mature cells. Now morphologically differentiated precursors such as myeloblasts, monoblasts, proerythroblasts and megakaryoblasts are produced.

These cells differentiate finally to produce mature granulocytes, red blood cells and platelets. [11]

The response of bone marrow to the transient physiological requirements is under the control of hematopoietic growth factors such as erythropoietin, granulocyte-macrophage colony stimulating factor (GM- CSF), granulocyte colony stimulating factor (G- CSF) and thrombopoeitin which acts through the receptors which are expressed on the committed progenitor cells having restricted potential for differentiation. [11]

The lymphoid committed precursors differentiate into pre cells, these precursors finally differentiate to produce mature B cells, T cells and Natural Killer (NK) cells. (Fig 1) [11]

FIGURE 1: HEMATOPOIESIS



BONE MARROW ARCHITECTURE:

Normal Anatomy:

Although bone marrow examination is an invasive procedure, it is still a simple and safe bedside procedure causing moderate discomfort to the patients with minimal bleeding. It is useful in evaluation in cases of unexplained cytopenias and malignant conditions like leukemia. [5]

Bone marrow aspiration and biopsy is valuable in assessment of bone marrow architecture, cellularity and distribution of cellular elements. A bone is made up of cortex and medulla. Cortex is the outer layer composed of solid compact bone. Medulla is an anatomizing network of trabecular bone; interstices of these trabeculae form the medullary cavity and contain the bone marrow. Bone marrow contains hematopoietic cells and are supported by fat cells, stromal cells, histiocytes, extracellular matrix and blood vessels. [14]

Distribution of Cellular Elements: [14]

Myeloid precursor cells: Endosteal and paratrabecular area

Maturing myeloid cells: Intertrabecular area

Erythroid series: Intertrabecular area

Megakaryocytes: Association with sinusoids

Fat cells: Particularly prominent adjacent to trabeculae

Lymphocytes: Scattered among Interstitium, sometimes may form small lymphoid nodule or follicle

Plasma cells: Associated with macrophages and commonly located around capillaries

Osteocytes: Within bony lacunae

Osteoblasts: Along the bone spicules or layer of osteoid

Osteoclasts: Multinucleated cells lying in hallow known as How ship's lacunae

BONE MARROW ASPIRATION:

Bone marrow aspiration is most commonly performed on the sternum and the ileum. In children up to the age of 18 months aspiration from the medial surface of tibia will provide useful diagnostic material. Bone marrow aspiration is extremely useful for detailed study of the morphology of cells. Also it permits the use of cytochemical stains and immunohistochemical markers, to study the cell markers by flow cytometry and for cytogenetic analysis in suspected cases of hematological neoplasms. [14]

Cytochemical stains: [14]

1. Perls' stain for iron: A Perls' or Prussian blue stain is used to demonstrate haemosiderin in the macrophages and within the erythroblasts of bone marrow.
2. Myeloperoxidase and Sudan B black staining: To identify cells showing granulocytic differentiation.
3. Non-specific esterase or combined esterase stain: To identify cells showing monocytic differentiation.

TREPHINE BIOPSY OF BONE MARROW:

Trephine biopsy can be easily carried out either on anterior or posterior iliac crest. Posterior approach is preferred to anterior. [14]

Trephine core biopsy specimens are suitable for histological examination and for imprint preparation. Touch preparations are important in cases where aspiration is not possible to obtain as it allows studying cytological details. Imprint preparation is made either by touching slide on biopsy core or rolling the core gently between the two slides. [14]

Adequate trephine biopsy should contain at least 5- 6 intertrabecular spaces and after processing should be at least 2-3cm in length. [14]

Bone marrow cellularity is assessed more accurately on biopsy sections, although it can be assessed from bone marrow aspiration material. Cellularity depends on the age and site of biopsy. Cellularity is expressed as a percentage of marrow cavity occupied by hematopoietic tissue. In neonates marrow is extremely cellular with negligible fat, and then cellularity decreases steadily with advancing age. An average decrease in marrow cellularity in the iliac crest from 64% in the second decade of life to 29% in the eighth decade of life has been reported. [14]

TABLE 1: HEMATOLOGICAL VALUES IN NORMAL ADULTS: [15]

(Expressed as a mean ± 2SD)

	Men	Women
Hemoglobin	15 ± 20 g/l	135 ± 15 g/l
Red blood cell (RBC) count	5.0 ± 0.5 x 10^{12} /l	4.3 ± 0.5 x 10^{12} /l
Mean cell volume (MCV)	92 ± 9 fl	92 ± 9 fl
Mean cell hemoglobin (MCH)	29.5 ± 2.5 pg	29.5 ± 2.5 pg
Mean cell hemoglobin concentration (MCHC)	330 ± 15 g/l	330 ± 15 g/l
Red cell distribution width (RDW - CV)	12.8 ± 1.2 %	11.6- 14%
White blood cell count	4.0- 10.0 x 10^{9} /l	4.0- 10.0 x 10^{9} /l
Platelet count	280 ± 130 x 10^{9} /l	280 ± 130 x 10^{9} /l
Reticulocyte count	0.5-2.5%	0.5-2.5%

TABLE 2: NORMAL RANGE OF DIFFERENTIAL COUNTS ON ASPIRATED MARROW: [13]

	OBSERVED RANGE (%)	MEAN (%)
NEUTROPHILIC SERIES (TOTAL)	49.2 – 65	53.6
• Myeloblasts	0.2-1.5	0.9
• Promyelocytes	2.1-4.1	3.3
• Myelocyte	8.2-15.7	12.7
• Metamyelocyte	9.6-24.6	15.9
• Band form	9.5-15.3	12.4
• Segmented	6.0-12.0	7.4
EOSINPHILIC SERIES (TOTAL)	1.2-5.3	3.1
• Myelocyte	0.2-1.3	0.8
• Metamyelocyte	0.4-2.2	1.2
• Band form	0.2-2.4	0.9
• Segmented	0-1.3	0.5
BASOPHIL AND MAST CELLS	0-0.2	<0.1
ERYTHROID SERIES (TOTAL)	18.4-33.8	25.6
• Pronormoblast	0.2-1.3	0.6
• Basophilic	0.5-2.4	1.4
• Polychromatophilic	17.9-29.2	21.6
• Orthochromatic	0.4-4.6	2.0
LYMPHOCYTE	11.1-23.2	16.2
PLASMA CELL	0.4-3.9	1.3
MONOCYTE	0-0.8	0.3
MEGAKARYOCYTE	0-0.4	<0.1
RETICULIN CELLS	0-0.9	0.3
MYELOID TO ERYTHROID RATIO	1.5-3.3	2.3

PANCYTOPENIA:

Pancytopenia is an important and common hematological disorder. Pancytopenia is decrease in the three major formed components of blood viz. red blood cells, white blood cells and platelets due to various disease processes. [4]

Red blood cells (RBCs), white blood cells (WBCs) and platelets are the essential cellular components of blood. The concentration of these cellular constituents is maintained in the blood within well defined limits, unless there is disturbance in the balance between the production and elimination due to pathologic process. [12]

Anemia is defined as "insufficient RBC mass to adequately deliver oxygen to peripheral tissues". For practical purposes, measurements any of the three concentrations of whole blood can be used to diagnose the presence of anemia: the hemoglobin (Hb), usually expressed as grams per deciliter, Hematocrit (Hct), RBC concentration in cells per microlitre.

Neutropenia is defined as an absolute neutrophil count less than 2SD below the normal mean for the age. In children of age 1 month to 10 years, an absolute neutrophil count of less than 1.5×10^9 /L, and for individuals older than 10 years of age a count less than 1.8×10^9 /L is considered. [16]

Thrombocytopenia is the presence of platelet count less than $150 \times \times 10^9$ /L. [16]

Pancytopenia is a hematological condition characterized by the simultaneous presence of anemia, leucopenia and thrombocytopenia. [12]

Several studies have previously sought to determine the etiology, clinical manifestations and bone marrow findings and thus the different treatment modalities of pancytopenia.

Etiology of Pancytopenia:

Pancytopenia results from various primary or secondary hematological and other non- hematological disorders affecting the bone marrow. Various diseases leading to Pancytopenia are as follows: [12, 13]

I) Hypocellular Bone Marrow

1. Acquired Aplastic Anemia
2. Inherited Aplastic Anemia
    - Fanconi Anemia
    - Dyskeratosis Congenita
    - Amegakaryocytic Thrombocytopenia
    - Shwachman -Diamond Syndrome
    - Reticular Dysgenesis
3. Hypoplastic Myelodysplastic Syndrome
4. Acute Leukemia in Hypoplastic Marrow (rare)
5. Lymphoma in Hypoplastic Marrow
6. Cytotoxic Agents and Radiotherapy

II) Cellular Bone Marrow with Primary Marrow Disorders

1. Acute leukemia
2. Lymphomas
3. Hairy Cell Leukemia
4. Myelodysplasia
5. Myelofibrosis
6. Paroxysmal Nocturnal Hemoglobinuria
7. Multiple Myeloma
8. Heamophagocytic Lymphohistiocytosis
9. Osteopetrosis

III) Cellular Marrow with Systemic Disorders

1. Hypersplenism
2. Nutritional Deficiencies
    - Vitamin B12 Deficiency
    - Folic Acid Deficiency
3. Alcoholism
4. Metastatic Solid Tumors
5. Autoimmune
    - Systemic Lupus Erythematosus
    - Sjogren Syndrome
6. Infections
    - Overwhelming Infection/Sepsis
    - Tuberculosis
    - Brucellosis
    - Kala- Azar
7. Storage Disorders:
    - Gaucher Disease
    - Niemann-Pick Disease
8. Sarcoidosis

ACQUIRED APLASTIC ANEMIA:

Aplastic anemia (AA) is a clinical syndrome resulting from a marked decrease in bone marrow cell production and causes anemia, granulocytopenia, monocytopenia, thrombocytopenia and reticulocytopenia. It is associated with hypocellular marrow without any abnormal or malignant cells or fibrosis of marrow.[17]

Etiologic spectrum of aplastic anemia is represented by exposure to drugs and chemicals like cytotoxic agents, non steroidal inflammatory drugs, chloramphenicol, antiepileptics, gold salts and benzene. Viral infections by Ebstein Barr Virus, Hepatitis Virus, Parvovirus, and Human Immunodeficiency Virus are common causes. Immunological diseases like Eosinophilic Fasciitis, Thymoma, Paroxysmal Nocturnal Hemoglobinuria, and Pregnancy are associated with Aplastic Anemia. [17]

Pathophysiologically, T- cell mediated organ specific destruction of hematopoietic cells due to excessive production of Interferon, Tumor Necrosis Factor, and Interleukin-2 has been described. [18]

Chemical or physical agents induce DNA damage and apoptosis by direct chemical toxicity and immune mediated destruction.[17, 19]

INHERITED APLASTIC ANEMIA:

FANCONI ANEMIA (FA):

Fanconi Anemia is an autosomal recessive disorder characterized by the presence of instability of chromosomes with variable clinical presentation, which includes congenital anomalies, progressive pancytopenia and susceptibility to various cancers. Fanconi Anemia patients have high risk of progression to MDS and AML.[20]

In the year 1972, Guido Fanconi reported the first case of "familial syndrome of pancytopenia and congenital physical abnormalities." It is usually diagnosed in children between the age group of 5- 15 years with equal distribution in both the sexes. Cells in Aplastic Anemia are hypersensitive to various oncogenic and mutagenic factors that determine chromosomal changes like rotations, angulations and fractures, with inability of DNA to recover after the break. [21]

The genes that are found to be mutated in FA are called FNAC genes. Till date, 16 different FNAC genes have been reported. Among them, FNACA are most frequently affected gene, which constitute about 60-65%.[22]

DYSKERATOSIS CONGENITA:

Dyskeratosis congenita is an X- linked inherited bone marrow failure syndrome characterized by the classic triad of 'reticular skin pigmentation, nail dystrophy and leukoplakia'. Clinical manifestations occur between 5 and 12 years of age. Most of the cases show X- linked recessive pattern of inheritance with mutation in DKC1, an X- linked genes, which leads to abnormally shortened telomeres and reduced telomerase activity. Consequently leads to premature aging of tissues with high replicative requirement. [23]

CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA (CAMT):

CAMT is a rare autosomal recessive inherited bone marrow failure syndrome, initially presents with severe thrombocytopenia, which can progress to evolve into Aplastic Anemia and Leukemia. The disorder presents in infancy with or without physical anomalies. [24]

CAMT is associated with mutation in the c- *Mpl* gene that encodes the thrombopoeitin (TPO) receptor. These patients will have high levels and functional serum TPO. [25]

SHWACHMAN- DIAMOND SYNDROME (SDS):

SDS is a multisystem autosomal recessive disorder, characterized by pancreatic exocrine dysfunction, dysostosis of metaphysis, and varying degrees of impaired hematopoiesis with cytopenias. One third of these patients have the risk of progression to MDS and Acute Myelogenous Leukemia. [26]

In majority (90%) of cases, mutations have been detected in the Shwachman – Bodian-Diamond syndrome gene, which is located on chromosome 7q11, encodes a SBDS protein and plays a role in ribosome biogenesis and in mitotic spindle stabilization. [27]

MYELODYSPLASTIC SYNDROME (MDS):

The myelodysplastic syndromes are group of neoplasms of the bone marrow characterized by the presence of ineffective hematopoiesis. This results in morphological dysplastic changes in hematopoietic cells of bone marrow and peripheral cytopenia(s) and propensity to progress to acute myeloid leukemia. In the previous classification cytopenia was essential for the diagnosis of MDS, but the 2016 revision to the WHO Classification of MDS stresses mainly on the degree of dysplasia and blast percentage for classification of the disease. As lineage specific dysplastic changes frequently do not correlate with specific peripheral cytopenia(s), the recent WHO classification removed the terms "Refractory Anemia" and "Refractory Cytopenia" and replaced these with "Myelodysplastic Syndrome" followed by appropriate findings such as single vs. multilineage dysplasia, ring sideroblasts, excess blasts or cytogenetic abnormalities the del(5q). Refractory cytopenia of childhood is unchanged as provisional entity in this classification. [28]

MDS usually affects during the middle age, with equal incidence in both sexes. Occurrence of disease before 50 years of age is rare except in cases with previous history of irradiation and chemotherapy. Shah NM *et al* in their study of an analysis of 30cases of MDS showed incidence of MDS in < 18 years of age (10% cases). [29]

There are studies to show evidence of both for and against the concept that MDS is a stem cell disorder, as it may occur more commonly in myeloid cell lineages, i.e. erythroid, granulocytic or monocytic and megakaryocytic lineages. Nilsson and colleagues, in their study showed 92- 100% of 5q- deletion in 11 patients of MDS with 5q genetic abnormality. They also detected 5q deletion in pro- B cells, isolated from the 5q- MDS patients showing the involvement of the lymphoid component. [30]

Previous studies showed that Interferon (IFN) signaling, TNFα signaling and TGFβ signaling and also oxidative DNA damage stress responses are associated with increased apoptosis and decreased proliferation and survival of early hematopoietic cell compartment. These studies lead to improvement in hematopoiesis in MDS by inhibiting TGFβ signaling.[30]

Bone marrow fibrosis has been reported in patients with MDS. [31]

Ustwani OA *et al* in their studies showed the association of autoimmune diseases with MDS. [32]

Study done by Copley GB *et al* (2017) reported potential risk of development of MDS with exposure to benzene, which was more commonly seen among the farm residents. They also showed that benzene also affects MDS cell types that are not predominantly of erythroid cell lineages. [33]

MYELOFIBROSIS:

Reticulin fibers are the component of bone marrow stromal environment and provide connective structure and support for hematopoietic progenitor cells.[32] Myelofibrosis is a condition characterized by an increase in the deposition of reticulin fibers and in some cases collagen fibers. [34]

Pathological increase in bone marrow fibrosis is seen in variety of benign and malignant conditions like several myeloid neoplasms, including myeloproliferative disorders (primary myelofibrosis, myelofibrosis secondary to thrombocythemia or polycythemia, BCR-ABL1+ chronic myelogenous leukemia), myelodysplastic/ myeloproliferative (MDS/MPN) disorders (chronic myelomonocytic leukemia, refractory anemia with ring sideroblasts and thrombocytosis) and acute leukemia (acute megakaryoblastic leukemia, acute pan-myelosis with myelofibrosis). [35]

PRIMARY MYELOFIBROSIS (PMF):

PMF is a clonal myeloproliferative disorder (MPD), characterized by variable degree of cytopenia(s) and /or cytosis, leucoerythroblastic blood picture, bone marrow fibrosis and extramedullary hematopoiesis associated with hepatosplenomegaly.[35]

Bone marrow fibrosis is mainly caused by abnormal number and/or function of megakaryocytes and platelets. It is due to cytokines from megakaryocytes and platelets and transforming growth factor (TGF), which is a potent stimulator of fibroblast collagen synthesis and plays an impotant role in determining increased deposition of bone marrow stromal fibrosis.[31]

More recently, a study done by Levine RL *et al* in 2005, identified *JAK2V617F* mutations in granulocyte DNA in 50% of patients with PMF.[31]

Pikman Y *et al* in their study demonstrated somatic mutation in the transmembrane region of MPL (*MPLW515L*) in a subset of JAK2V617F-negative MF. Patients with the *MPLW515L* mutation will also be sensitive to inhibition with small molecule JAK2 inhibitors. [36]

Papadantonakis N *et al* in their study highlighted the role of protein lysyl oxidase (LOX) in the proliferation of megakaryocytes, ploidy and deposition of fibers. [37]

PMF presents in two stages. [28]

- Pre primary Myelofibrosis
- Overt primary Myelofibrosis

WHO Criteria for Pre PMF [28]

Major Criteria:

1. Megakaryocytic proliferation and atypia, without reticulin fibrosis grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis.
2. Not meeting the WHO criteria for BCR-ABL1^{+} CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms.
3. Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker, or absence of minor reactive BM reticulin fibrosis.

Minor Criteria:

Presence of at least 1 of the following, confirmed in 2 consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis $>11 \times 10^9/L$
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range

Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion

WHO Criteria for Overt PMF: [28]

Major Criteria:

Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3

1. Not meeting WHO criteria for ET, PV, BCR-ABL1^{+} CML, myelodysplastic syndromes, or other myeloid neoplasms
2. Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker, or absence of reactive myelofibrosis.

Minor Criteria:

Presence of at least 1 of the following, confirmed in 2 consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukcocytosis $>11 \times 10^{9}/L$
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis

Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

TABLE 3: Grading of Myelofibrosis: [28]

MF- Grade 0	Scattered linear reticulin without intersections (crossovers) corresponding to normal BM
MF- Grade1	Loose network of reticulin fibers with many intersections, especially in perivascular areas
MF-Grade 2	Diffuse and dense increase in reticulin fibers with extensive intersections, with focal bundles of thick fibers mostly consistent with collagen, and/or focal osteosclerosis
MF-Grade 3	Diffuse and dense increase in reticulin fibers with extensive intersections and coarse bundles of thick fibers collagen, usually associated with osteosclerosis

International working group for myeloproliferative neoplasms research and treatment in 2009 proposed a dynamic prognostic model to predict survival in patients with primary Myelofibrosis. [38]

TABLE 4:Prognostic model for PMF. [38]

Prognostic variable	**Value**		
	0	1	2
Age in years	≤65	≥65	
WBC count, $x10^9$/L	≤25	>25	
Hb, g/dL	≥10		<10
Peripheral blood blast count, %	<1	≥1	
Constitutional symptoms: Yes/No	No	Yes	

As low: 0; intermediate -1: 1 or 2; intermediate-2: 3 or 4; and high: 5 or 6.

PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA:

Paroxysmal Nocturnal Hemoglobinuria (PNH) is an acquired clonal disorder of stem cells of one or the several hemopoietic stem cells characterized by the presence of intravascular hemolysis, nocturnal hemoglobinuria, and thrombosis of veins and are associated with failure of bone marrow. [39]

PNH results from X- linked PIGA gene somatic mutation in HSC and its mature progeny, resulting in defective biosynthesis of the glycosylphoshpatidylinositol (GPI) molecules, which serves as anchor to various cell membrane proteins. [40]

Intravascular hemolysis is due to an increased susceptibility to complement-mediated lysis of PNH red cells, as these cells lack GPI anchored proteins CD55and CD59 on their membranes. CD55 and CD59 protect the cell lysis from compliments.[41]

PNH has been recognized by two presentations. One form, predominantly hemolytic without overt marrow failure, was classified as classic PNH; and the other, with marrow failure often described as Aplastic Anemia PNH Syndrome. [42]

Maciejewski JP *et al* (2001) demonstrated the presence of glycophosphatidyl inositol-anchored protein-deficient clones in Aplastic Anemia patients, hence suggested relationship between PNH and Aplastic Anemia. [43]

LEUKEMIA:

Acute Lymphoblastic Leukemia is the most prevalent malignancy during childhood. Continuous proliferation of malignant white blood cells results in excess of blasts both in the marrow and peripheral blood, which makes its diagnosis easy. But many times acute lymphoblastic leukemia may present initially with pancytopenia and a hypoplastic marrow leading to the initial diagnosis of Aplastic Anemia. [44]

Villarreal-Martínez L *et al* [45] and Breatna F *et al* [46] in their study showed rare occurrence of ALL in case of prolonged Aplastic Anemia in young children.

Kulkarni KP *et al* in their study compared 83 cases of ALL who presented with pancytopenia with those cases of ALL without pancytopenia. They compared the survival of patients, prognostic factors in these two groups and concluded that patients with pancytopenia have better survival rate compared to the other group. [47]

In AML, patients typically present with Anemia, Neutropenia and /or Thrombocytopenia. Total leucocyte count is variable, ranging from leucopenia to leukcocytosis with increase in blast count. Presentation with pancytopenia results from impaired hematopoiesis due to replacement or suppression of normal marrow elements by malignant blasts. [48]

Hypocellular AML occurs in 5-12% of all cases of AML. It is typically seen in adults. Diagnosis of AML in hypocellular marrow poses a challenge to distinguish between hypocellular MDS and Aplastic Anemia, as these share common features including cytopenia and dysplasia. [49]

Bennett JM, Orazi A, in 2009, proposed diagnostic criteria and a standardized approach to differentiate between hypocellular MDS, hypocellular AML and Aplastic Anemia. Along with examination of bone marrow aspiration and bone marrow biopsy with special stains like Perls' stain and reticulin stain. They recommended additional studies including selective immunohistochemistry, flow cytometry and cytogenetics.[50]

Jain D, Singh T, reported a rare case of hypocellular AML, as a rare cause of bone marrow necrosis. [51]

MULTIPLE MYELOMA:

Multiple myeloma (MM) is a neoplasm of plasma cells characterized by the clonal proliferation of neoplastic plasma cells in the bone marrow with increased serum and/or urine M- protein. Multiple myeloma presenting initially as pancytopenia is rare. [52]

Shridevi HB *et al* [52] studied cases of Multiple Myeloma presenting with pancytopenia, a detail study included peripheral smear examination, bone marrow aspiration study and serum electrophoresis.

MEGALOBLASTIC ANEMIA:

Megaloblastic anemia is so named because of presence of morphologically abnormal erythroid precursors, which were labeled as megaloblasts by Ehrlich in 1980. Megaloblastic anemia is characterized by distinctive cytological and functional abnormalities in peripheral blood and bone marrow cells due to impaired DNA synthesis. [53]

Most common causes of megaloblastic anemia worldwide are cobalamin and folate deficiency. Due to deficiency of these, there is failure of folate dependent conversion of dUMP to dTMP. Deoxyuridine triphosphate (dUTP) levels in place of deoxythymidine triphosphate become abundant and become incorporated into the DNA of folate deficient cells. This abnormal incorporation affects the DNA excision repair mechanism, resulting in DNA strand breaks, fragmentation and apoptotic cell death. [54]

Vitamin deficiency has a broad spectrum of etiological factors such as inadequate dietary intake, impaired absorption either due to intrinsic factor deficiency (pernicious anemia) and/or generalized malabsorption conditions. Amongst these, inadequate dietary intake is the most common cause of deficiency. [55]

1964 Haltersley *et al* Davidson (1971), Mcphedran (1973), Davidson (1978) and Colon Otero et al (1992), studied various causes of macrocytic anaemia and observed Megaloblastic Anemia, Liver Cirrhosis and Alcohol abuse as the most common causes.[56]

IRON DEFICIENCY ANEMIA:

Iron deficiency is the most common cause of nutritional deficiency anemia both in developed and developing countries. Leucopenia has been found in some patients of iron deficiency anemia, but the overall distribution of leucocyte count appears to be approximately normal. More commonly iron deficiency anemia associated with thrombocytosis and thrombocytopenia. [57]

Iron deficiency anemia presenting as pancytopenia is very rare. Jhamb R. and Kumar A. presented a case of iron deficiency anemia with pancytopenia, which worsened with initiation of treatment. [58]

ALCOHOL INDUCED PANCYTOPENIA:

In 1955 Jandls postulated the theory of bone marrow suppression as a direct consequence of excessive and prolonged ingestion of alcohol. This was supported by Sullivan and Herbert in 1963, that excess of ethanol could indeed suppress hematopoiesis in man. Ballard HS *et al* reported a case of alcohol associated pancytopenia with bone marrow hypoplasia. [59] Many studies have suggested that alcohol acts as a marrow toxin. Suppression of hematopoiesis is a common hematological complication of alcohol consumption. [60]

In 1987, Weston CF *et al* reported a case of pancytopenia and folic acid deficiency in alcoholic patients. Folic acid deficiency is the most common nutritional

defect seen in alcoholics and is related to various factors. It has been studied that alcoholics often ingest folate deficient diet and also suggested that there is reversible sequestration of folate within hepatocytes and acute interruption of enterohepatic circulation of methyltetrahydrofolate. Acute folate deficiency leading to megaloblastic hematopoiesis, sideroblasts and vacuolization of pronormoblasts and promyelocytes and life threatening pancytopenia, and is reversible with folic acid supplementation. [61]

AUTOIMMUNE DISORDERS CAUSING PANCYTOPENIA:

SYSTEMIC LUPUS ERYTHEMATOSIS (SLE):

The hematological manifestations of Systemic Lupus Erythematosis commonly include anemia, which is often autoimmune hemolytic anemia; leucopenia and thrombocytopenia. It is also associated with recurrent arterial and venous thrombosis due to anti-phospholipid antibodies (APLA). Occurrence of pancytopenia in SLE is rare and is attributed to immune mediated peripheral destruction of cells. Jose W. *et al* [62] presented a rare case of Aplastic Anemia in a case of SLE.

Etiology of Aplastic Anemia is autoimmune in nature; this has been proved by many studies. Both compliment dependent and independent auto antibodies were found to suppress formation of erythroid and granulocyte colony by colony forming units. Autoreactive T cells were also shown to cause damage to hematopoietic stem cells through direct cytotoxic destruction or by inducing apoptosis. [62]

Voulgarelis M. *et al* reported the bone marrow findings in 40 patients with SLE, which include hypocellularity, necrosis of marrow and stromal changes like edema, fibrosis and vascular changes. [63]

SJOGREN SYNDROME:

Primary Sjogren Syndrome is an autoimmune disorder characterized by the presence of xerostomia and xerophthalmia without evidence of any other systemic autoimmune disease. Association of Aplastic Anemia with Sjogren syndrome is rare. Quiquandon I. *et al* reported a case of Aplastic Anemia with Sjogren syndrome, with increase in γ-δ TCR+ cells, which have cytotoxic function, suggesting the possible involvement of γ-δ TCR+ cells in the pathogenesis of Aplastic Anemia. [64]

INFECTIOUS CAUSES OF PANCYTOPENIA:

MALARIA:

Malaria is a parasitic infection caused by obligate intracellular protozoa of Plasmodium genus. In humans, malaria is caused by the four species viz. P. vivax, P. ovale, P. malariae, and P. falciparum. These are typically transmitted by the female *Anopheles* mosquito or rarely through blood transfusion and transplacental transmission.[65]

Destruction of red blood cells by the parasite lead to hemolytic anemia can manifest as anemia, fatigue and hemodynamic disturbances. Thrombocytopenia is a common manifestation in malaria. It is immune mediated and in severe infections disseminated intravascular coagulation can lead to thrombocytopenia. [65]

In malaria, the mechanism of thrombocytopenia is not clearly known. Immune mediated lyses, sequestration in the spleen and dyspoietic process in the marrow with diminished platelet production have been postulated. [66]

Vinoth PN, *et al* in 2011, reported a case of heamophagocytic syndrome (HPS) with P. falciparum infection. Both falciparum and vivax infections have been

reported to cause HPS; however, vivax being a rare cause. Heamophagocytic syndrome may play an important role in the pathogenesis of pancytopenia which is observed in malarial infestation. [67]

BRUCELLOSIS:

Brucellosis is primarily a contagious disease of domestic animals. In acute brucellosis, hematological manifestations such as anemia and leucopenia are commonly seen. Pancytopenia is a rare occurrence. EL Koumi MA, *et al* [68] studied 60 cases of brucellosis with antibody titre >1/160 and confirmed on blood or bone marrow culture and showed 18.3% incidence of pancytopenia in these patients.

Possible mechanisms suggested for occurrence of pancytopenia in brucellosis are hypersplenism, formation of granuloma in bone marrow and phagocytosis of formed elements by reticuloendothelial cells or bone marrow suppression due to associated septicemia.[68]

TUBERCULOSIS:

Tuberculosis is still a major health problem in developing countries. Miliary tuberculosis is a condition resulting from hematogenous dissemination of tubercle bacilli from an established focus to various organs of the body. Hematological abnormalities like anemia, leucopenia, leukcocytosis, monocytosis and thrombocytopenia due to tuberculosis are common. [69]

Deep HS *et al* [69], Avasthi R *et al* [70], Yadav TP *et al* [71] in their study showed rare occurrence of pancytopenia in a case of military tuberculosis.

Several causative mechanisms were considered for the development of pancytopenia in disseminated and extra pulmonary tuberculosis like hypersplenism, maturational arrest, and caseating and non-caseating granulomas infiltrating the bone marrow causing reversible or irreversible fibrosis of bone marrow. [72]

HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION:

Cytopenias are among the most common hematological complications of HIV. All the lineages of blood are involved; anemia being the most common manifestation. The main cause for anemia of chronic disease is due to disordered cytokine homeostasis in the bone marrow. HIV is cytotoxic to T- helper lymphocytes. These infected T- helper cells directly inhibit the development of bone marrow progenitor cells and thus suppress hematopoiesis. Infection of monocytes in the marrow leads to alteration in the release of cytokines, which indirectly suppresses the response capacity of hematopoietic stem cells to anemia or peripheral cytopenia. [73]

In HIV patients, opportunistic infections with M. tuberculosis, M. avium complex and cryptococcus neoformans infiltrate the bone marrow with formation of reactive granulomas can cause cytopenias. [73]

Dikshit B. *et al* studied 200 cases of HIV. In their study, anemia was most common hematogical finding which inversely correlated with CD4 counts. Bone marrow evaluation was done for cases of pancytopenia of which 6 cases showed granulomas and 3 cases showed HPS. [74]

A common complication of HIV infection of the bone marrow is dyspoietic hematopoiesis known as HIV - myelopathy. Compared to a true myelodysplastic syndrome, HIV- myelopathy is not considered as a true stem cell disorder, rather it represents spectrum of morphological changes secondary to HIV infection or HAART. This theory has been supported by low rate of progression of HIV myelopathy to acute myelogenous leukemia, unlike cases of true non HIV related myelodysplasias. Zota V. *et al* reported erythroleukemia (FAB M6b), in an HIV positive patient, presented with pancytopenia.[75]

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH):

Heamophagocytic lymphohistiocytosis (HLH) is a potentially severe hyperinflammatory condition caused by exaggerated but ineffective immune response. Heamophagocytic lymphohistiocytosis can be classified in to primary (genetic) or secondary (acquired) according to the underlying etiology. [76]

Primary HLH is inferred due to defective termination of immune responses which lead to persistent macrophages and cytotoxic T cells stimulation and activation. It is also postulated that, there is persistent activation of immune effector cells which fail to remove antigens. EBV is the most common cause of infection associated HLH. Secondary HLH occur with other severe infections, malignant conditions, rheumatologic disorders and metabolic disorders. [76]

Diagnosis of HLH can be established either by a molecular diagnosis consistent with HLH.

Also the presence any five of the following eight criterion establishes the diagnosis of HLH. [76]

- Fever
- Splenomegaly
- Cytopenia affecting 2-3 lineages in peripheral blood
- Hypertriglyceridemia
- Hypofibrinogenemia
- Hemophagocytosis in bone marrow, spleen or lymphnodes
- Low or absent NK cell activity
- Hyperferritenemia
- High level of soluble interleukin 2 receptor

HYPERSPLENISM:

Hypersplenism is broadly defined as "sequestration and/or destruction of blood cells occurring in an enlarged spleen, associated with peripheral cytopenia including anaemia and/or neutropenia and /or thrombocytopenia". [77]

Hypersplenism can occur as a primary event due to an unknown pathogenic stimulus. Some of the important causes of secondary hypersplenism are haematological malignancies, storage disease, infections like malaria, typhoid, brucellosis, leishmaniasis, collagen vascular diseases, congestive splenomegaly and splenic tumours. [77]

STORAGE DISORDERS:

Glycolipid storage diseases are hereditary conditions occurring due to the deficiency of lysosomal enzymes in which specific lipids get stored in single or multiple tissues and become enlarged. [78]

GAUCHERS DISEASE:

In 1882, P. C. E. Gaucher, described about the Gauchers disease for the first time. It is an autosomal recessive disorder caused by deficiency of a lysosomal enzyme, glucocerebrosidase, resulting in accumulation of glucocerebroside in macrophages and causes increased cell size and cytoplasmic striations, leading to formation of 'Gauchers cell'. [78]

Blood picture in Gauchers disease is moderate normocytic normochromic anemia. In some cases anemia may be severe. Mild to moderate leucopenia and thrombocytopenia are also common. Diagnostic feature is the existence of Gaucher cells in the marrow aspiration and splenic reticuloendothelial cells. These cells are large round to polyhedral with a diameter of 20-40µm or more, small and

eccentrically placed nucleus having pale and fine wavy fibril pattern of cytoplasm (tissue paper appearance of reticuloendothelial cells). [78]

NIEMANN-PICK DISEASE:

In 1914, Niemann reported a case of an infant who died at an age of 18 months with a disorder which was typical for Gauchers disease because of its early onset and rapid course. In 1922, Pick identified this disorder of rapid and progressive neurodegeneration in infants and identified that spingomyelin is the predominant phospholipid accumulating in this disease. [79]

Niemann- Pick disease is an autosomal recessive group of disorders in which spingomyelin storage occurs. The haematological findings in Niemann- Pick disease include thrombocytopenia and anemia. Splenic enlargement is also a common presenting feature and sequestration of cells in the spleen is thought to be the underlying cause of low haemoglobin, low platelets and leucopenia. [80]

Primary cellular site of pathology is the monocyte - macrophage system and the characteristic pathological cells are referred to as Niemann- Pick cells, which in general, resemble those of Gaucher cells but filled with small hyaline droplets, giving a honeycomb appearance. [80]

MALIGNACIES ASSOCIATED WITH PANCYTOPENIA

Bone marrow failure has been reported to be due to necrosis in neoplastic conditions. Bone marrow necrosis has been proposed to be secondary to ischemic injury due to hypoxia, obstruction of blood supply and thrombosis as a result of disseminated intravascular coagulation (DIC). [81]

Knupp C. *et al* [81] came across two patients with neoplasia who had extensive areas of necrosis in the marrow, without any evidence of sepsis or prior treatment of

malignancy, and subsequently developed fibrosis. It was associated with activation of TNF. This study suggested that cancer stimulates mononuclear cells to produce large amount of TNF which results in necrosis of the marrow.

Patients with malignancies commonly have anaemia, sometimes with other cytopenias. Cancer associated anaemia can be a consequence of invasion of the bone marrow, or due to therapy related suppression or from other underlying etiologies in the patient. Evaluation of laboratory tests along with the bone marrow examination can be helpful to arrive at a definitive diagnosis.[81]

Hemopoietic malignancies are the principal cause of one or more peripheral cytopenias. Malignant proliferation of cells suppresses growth of hematopoietic stem cells. The anaemia which is present is commonly macrocytic and associated with a decrease in reticulocyte count and clonal proliferation of erythropoiesis. While the diagnosis can be made with peripheral smear evaluation, Bone marrow aspiration and biopsy studies are often required for confirmation of diagnosis. [81]

Solid tumour metastasis to the bone marrow is common and is usually seen as advanced manifestation of malignancies. Among them most common solid tumours which metastasise to the bone marrow include Non Hodgkin Lymphomas, neuroblastoma in childhood, breast cancers, carcinoma prostate and rarely lung cancers. Satya V *et al* reported a case of non small cell lung carcinoma, in which patient presented with pancytopenia. [82]

EVALUATION OF PATIENTS WITH PANCYTOPENIA:

Presenting symptoms in pancytopenia are usually attributable to anemia, leucopenia or thrombocytopenia. [2]

Life span of RBCs is much longer than that of platelets and neutrophils. Hence anemia develops slowly and typical symptoms of anemia include weakness, fatigability, puffiness of face, edema, laxity, intolerance to strenuous work. Platelets are the first to get affected. Patients commonly present with mucocutaneous bleeding which is typical of thrombocytopenia. Sometimes other bleeding manifestations due to thrombocytopenia include epistaxsis, hematuria, GI bleeding, menorrhagia and rarely intracranial bleeding. After platelets the next to be affected is the myeloid series. It usually presents with neutropenia. The manifestations of neutropenia are throat infection, chest or soft tissue infections due to commensal organisms. Sometimes, pancytopenia patients may develop severe life threatening septicemia without any alarming local signs of underlying infections. [83] Hence pancytopenia can present with striking feature of many serious and life threatening illnesses.

The causes of pancytopenia are numerous. Underlying causes of pancytopenia are different in children compared to adults. Particular attentiveness should be given to the patient's age, significant family and past history. History of previous episode of pancytopenia, aplastic anaemia, inherited bone marrow failure syndromes (IBMFS), early fetal loss, history of malignancies, metabolic disorders, liver disease, or connective tissue disorders are of prime importance in such patients. [84]

PHYSICAL EXAMINATION: [12]

- Lymphadenopathy
- Hepatomegaly and /or splenomegaly
- Bone tenderness, deformity or tumor, Gum hypertrophy
- Signs of the underlying disorder causing hypersplenism
- Evidence of primary malignancies with or without metastases

LABORATORY INVESTIGATIONS:

ESSENTIAL INVESTIGATIONS IN ALL CASES:

- Peripheral smear examination
- ESR
- Bone marrow study; aspiration and trephine biopsy.

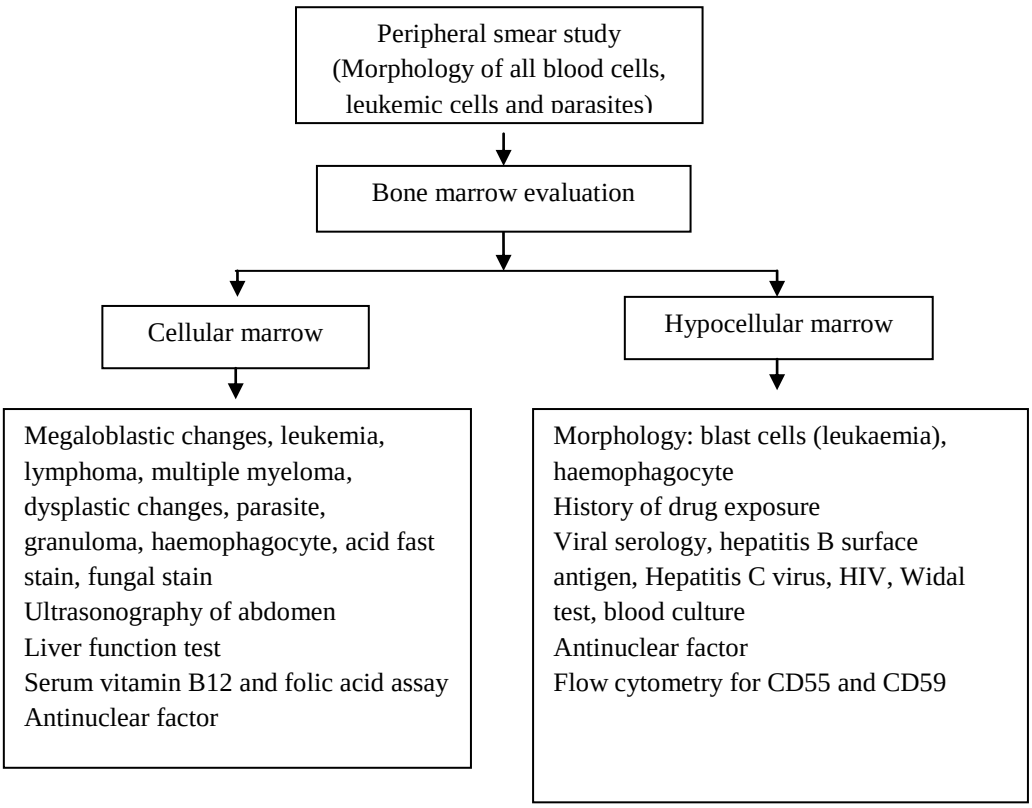
SPECIFIC INVESTIGATIONS:

- Bone X – ray (MM, metastatic carcinoma, lymphomas)
- Chest X – ray (tuberculosis, carcinoma , lymphomas)
- Serum protein electrophoresis (MM , Macroglobulinemia)
- Serum alkaline and acid phosphatase level (metastatic carcinoma)
- DNA antibody , lupus erthryomatous cell test (SLE)
- Urinary Bence – Jones Protein (MM)
- Needle biopsy of liver (hyperspleenism, lymphomas , disseminated tuberculosis)

Santra G. and Das BK. studied 111 adult pancytopenia patients. In their study they categorized various causes according to bone marrow cellularity. Most common cause of pancytopenia was aplastic anemia followed by hypersplenism and kala- azar.

Depending on the etiological spectrum they proposed algorithm for investigation of pancytopenia. [6] (Fig 2)

FIGURE 2:


Peripheral smear study
(Morphology of all blood cells, leukemic cells and parasites)
Bone marrow evaluation
Cellular marrow
Hypocellular marrow
Megaloblastic changes, leukemia, lymphoma, multiple myeloma, dysplastic changes, parasite, granuloma, haemophagocyte, acid fast stain, fungal stain
Ultrasonography of abdomen
Liver function test
Serum vitamin B12 and folic acid assay
Antinuclear factor
Morphology: blast cells (leukaemia), haemophagocyte
History of drug exposure
Viral serology, hepatitis B surface antigen, Hepatitis C virus, HIV, Widal test, blood culture
Antinuclear factor
Flow cytometry for CD55 and CD59

SOURCE OF DATA:

A cross sectional, hospital based study was carried out on patients fulfilling the inclusion and exclusion criteria attending either outpatient or inpatient departments referred to the Department of Pathology in BLDEU'S Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura. The study was conducted from 1st November 2015 to 30th June 2017.

INCLUSION CRITERIA:

1) Patients of all age groups
2) Cases showing the parameters as
    - Hb <10g /dl
    - WBC count < 4000 cells /cmm
    - Platelet < 150000 cells / cmm

EXCLUSION CRITERIA:

- Patients on myelotoxic chemotherapy were excluded.

METHODS OF COLLECTION OF DATA:

The study was carried out in patients of pancytopenia. In all cases appropriate history - bone pain, fever, night sweats, malaise, weight loss, pruritis, etc. and physical examination findings - hepatomegaly, splenomegaly, lymphadenopathy and sternal tenderness were noted. (Annexure III) Following investigations were carried out.

1. Complete blood count
2. Peripheral smear study

3. Reticulocyte count
4. Bone marrow study

SAMPLE COLLECTION:

2 ml of blood was collected by venipuncture under aseptic precautions in a bulb containing ethylene di-amine tetra acetic acid (EDTA). Samples were processed on automated hematology analyzer (SYSMEX XN-1000) and blood counts with other details were obtained.

PERIPHERAL SMEAR STUDY:

Peripheral smears were prepared, the films were air dried, and stained with Leishman's stain. Smears were examined under microscope for the following features:

- RBC morphology - to type morphological anemia, immature RBC's, any inclusions
- WBC morphology – for differential count, morphology of each cell, immature cells
- Platelet count and its morphology
- Any parasites

RETICULOCYTE COUNT:

Reticulocyte count was done using new methylene blue stain. The Normal Reticulocyte Count in adults is 0.5 – 2.5%

BONE MARROW ASPIRATION:

Bone marrow aspiration was performed in all the patients using Salah needle (Fig. 3) after obtaining written consent (Annexure II) for the procedure either from the patient or the guardian.

Slides were thoroughly dried and fixed in methanol for 20 minutes, stained with Leishman stain and marrow aspiration smears were examined for:

i) Cellularity
ii) M: E ratio
iii) Erythropoiesis
iv) Myelopoiesis
v) Megakaryopoiesis
vi) Others – plasma cells, lymphocytes, mast cells
vii) Parasites
viii) Abnormal cells

Special stains like Prussian blue stain were done to assess iron stores, and grading was carried out.

TABLE 5: BONE MARROW IRON GRADING

Grade	Criteria	Interpretation of iron stores
0	No iron granules observed	Absent
1+	Small granules in reticulum cells(seen only in oil immersion)	Normal
2+	Few granules visible under high power field	Normal
3+	Numerous small granules in all marrow particles	Normal
4+	Large granules in small clumps	Moderately increased
5+	Dense ,large clumps of granules	Markedly increased
6+	Very large deposits obscuring marrow detail	Markedly increased

Other special stains were done wherever required.

BONE MARROW BIOPSY:

Bone marrow biopsy was done using Jamshidi needle (Fig. 4) from posterior superior iliac spine.

Processing of tissue:

Decalcification was done using 9.5% of nitric acid in 1% EDTA. Then the tissue was processed using routine histological processing technique. Routine hematoxylin and eosin staining was done. Special stain like Reticulin stain was done wherever necessary.

Reporting:

Slides were examined and reported as follows

- Adequacy of biopsy.
- Cellularity and topography.
- Any abnormality

Compiling clinical details, hematological parameters and bone marrow study, the cases were studied. The cause for pancytopenia, age and sex distribution and other relevant details were noted and analyzed.

Total 120 cases of pancytopenia were studied. Sex distribution, distribution among various age groups, presenting complaints, physical findings, bone marrow findings and distribution of various causes pancytopenia were analyzed.

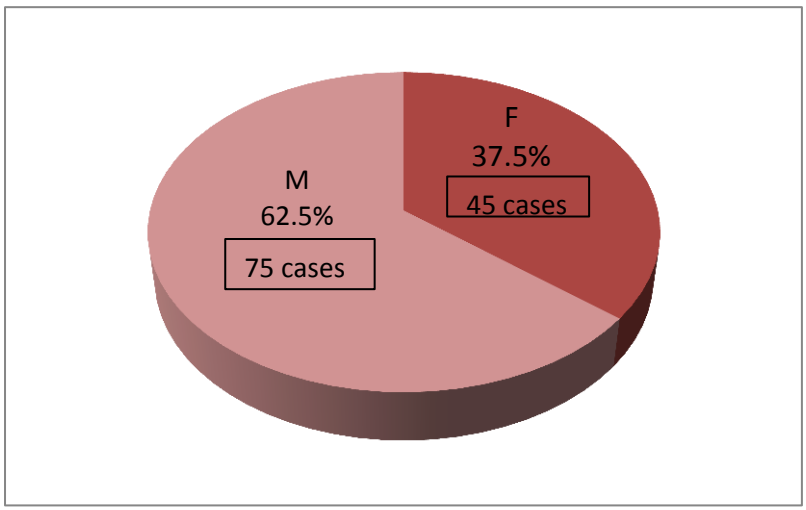
SEX DISTRIBUTION:

Out of total 120 patients studied, 75 (62.5%) were male and 45 (37.5%) were female with male to female ratio being 1.66:1. Our study shows male preponderance of pancytopenia.

TABLE 6: SEX DISTRIBUTION

Sex	**Frequency**	**Percentage (%)**
Male	75	62.5
Female	45	37.5
Total	**120**	**100**

FIGURE 5: PIE CHART SHOWING SEX DISTRIBUTION



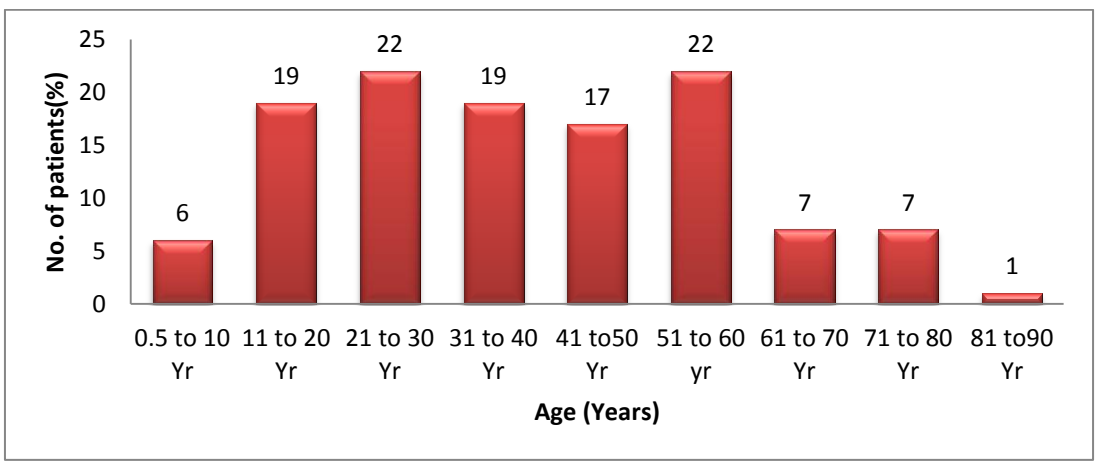
AGE DISTRIBUTION:

In our study, the age of patients ranged from 1 to 85 years (mean age of 42.5 years). Out of 120 cases, pancytopenia was observed in 19 pediatric patients (1-18 years); with 14 females and 5 males. Maximum number of cases were seen in 3rd (18.33%) and 6th decades (18.33%) followed by 2nd (15.83%) and 4th decades (15.83%).

TABLE 7: INCIDENCE OF PANCYTOPENIA IN DIFFERENT AGE GROUPS

Age group	No. of cases	Percentage
1-10	6	5
11-20	19	15.83
21-30	22	18.33
31-40	19	15.83
41-50	17	14.16
51-60	22	18.33
61-70	7	5.83
71-80	7	5.83
81- 90	1	0.83
Total cases	**120**	**100**

FIGURE 6: INCIDENCE OF PANCYTOPENIA IN DIFFERENT AGE GROUPS



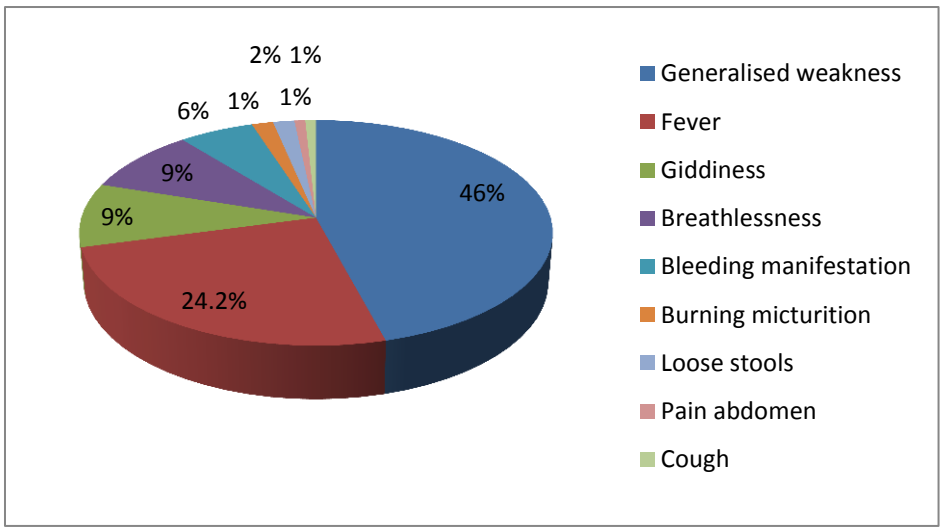
PRESENTING COMPLAINTS:

The most common mode of presentation was generalized weakness (46%), followed by fever (24.2%). Other presenting symptoms were giddiness, breathlessness, bleeding manifestation which include per rectal bleeding, bleeding gums, epistaxsis, per vaginal bleeding, burning micturition, loose stools, pain abdomen and cough.

TABLE 8: PRESENTING COMPLAINTS

Presenting Complaints	No. of cases	Percentage
Generalized weakness	57	46.7
Fever	29	24.2
Giddiness	11	9.17
Breathlessness	11	9.17
Bleeding manifestations	7	5.83
Burning micturition	2	1.67
Loose stools	1	0.83
Pain abdomen	1	0.83
Cough	1	0.83

FIGURE 7: PRESENTING COMPLAINT



PHYSICAL FINDINGS:

On physical examination pallor was present in all cases (100%). Lymphadenopathy and sternal tenderness was present in acute leukemia case. Hepatomegaly was present in 14 cases (11.6%) and splenomegaly in 18 cases (15%). Hepatosplenomegaly were seen in cases of megaloblastic anemia followed by iron deficiency anemia and leukemia.

TABLE 9: PHYSICAL FINDINGS

Physical findings	No. of cases (%)	Percentage (%)
Pallor	120	100
Icterus	29	24.16
Edema	8	6.7
Lymphadenopathy	1	0.83
Sternal tenderness	1	0.83
Hepatomegaly	14	11.6
Splenomegaly	18	15.0

HEMATOLOGICAL PARAMETERS:

Hemoglobin: The hemoglobin concentration ranged from 1.7 gm/dl to 10gm/dl. Maximum number of patients had hemoglobin levels between 6.1 to 10 gm/dl. The lowest level of hemoglobin seen in a case of Aplastic Anemia.

Total leucocyte count (TLC): The total leucocyte counts ranged from 500- 4000 cells/cmm. Majority of cases had total leucocyte count between 2500- 4000 cells/cmm. The lowest TLC was seen in a case of Megaloblastic Anemia.

Platelet count: The platelet counts ranged from 4000- 150000 cells/cmm. Maximum number of cases had platelet count between 4000- 50000 cells/ cmm. Lowest platelet count was seen in case of Megaloblastic Anemia.

Reticulocyte count: The reticulocyte count ranged 0.1- 10%. Majority of cases had a reticulocyte count between 0.1 and 1.5%. Low reticulocyte count was seen in case of Megaloblastic Anemia.

TABLE 10: HEMATOLOGICAL PARAMETERS

Hematological parameters	**Range**	**No. of cases**	**(%)**
Hemoglobin (g/dl)	1.7- 4	34	28.33
	4.1- 6	41	34.17
	6.1-10	**45**	**37.50**
Total leucocyte count (cells/cumm)	500- 1000	3	2.50
	1001- 2500	40	33.33
	2501- 4000	**77**	**64.17**
Platelet count (cells/cumm)	**4000- 50000**	**53**	**44.20**
	50001- 100000	47	39.20
	100001- 150,000	20	16.20
Reticulocyte count (%)	**0.1- 1.5**	**91**	**75.83**
	1.6- 2.5	24	20
	> 2.5	5	4.17

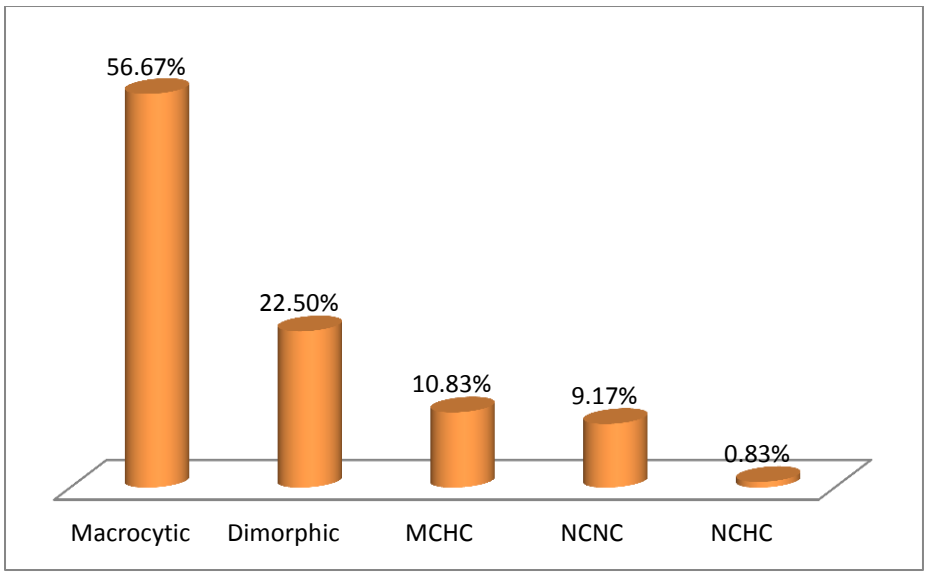
RBC MORPHOLOGY ON PERIPHERAL SMEAR:

On peripheral smear examination, the predominant red blood cell morphology was macrocytic in 68 cases (56.67%), followed by dimorphic and normocytic normochromic. 1 case (0.83%) showed normocytic hypochromic cells.

TABLE 11: RBC MORPHOLOGY ON PERIPHERAL SMEAR.

RBC morphology	**No. of cases**	**Percentage (%)**
Macrocytic	**68**	**56.67**
Dimorphic	27	22.50
MCHC	13	10.3
NCNC	11	9.17
NCHC	1	0.83
Total	120	100

FIGURE 8: RBC MORPHOLOGY ON PERIPHERAL SMEAR



Peripheral smear showed hypersegmented neutrophils and nucleated RBCs in cases of megaloblastic anemia. Immature WBCs were present predominantly in acute leukemia cases and few cases of megaloblastic anemia. Ring forms of P. falciparum malaria were present in one case.

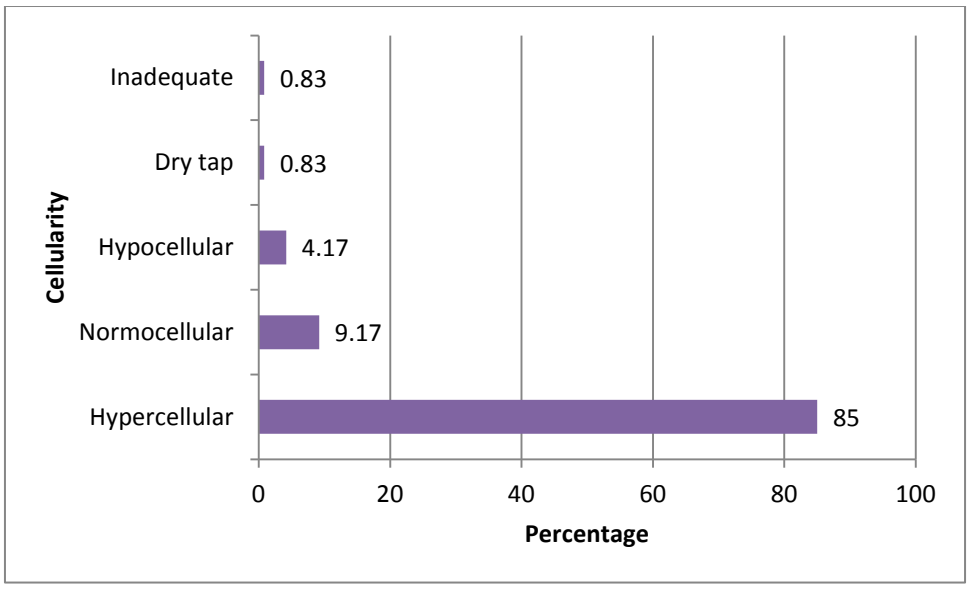
BONE MARROW CELLULARITY:

In present study, marrow was hypercellular in majority of the cases i.e. 102 cases (85%). Hypercellular marrow was the predominant feature of megaloblastic anemia. Hypocellular marrow was present in 5 cases.

TABLE 12: BONE MARROW CELLULARITY IN PANCYTOPENIA:

Cellularity	No. of cases	%
Hypercellular	**102**	**85**
Normocellular	11	9.17
Hypocellular	5	4.17
Inadequate	1	0.83
Dry tap	1	0.83
Total	120	100

FIGURE 9: BONE MARROW CELLULARITY IN PANCYTOPENIA



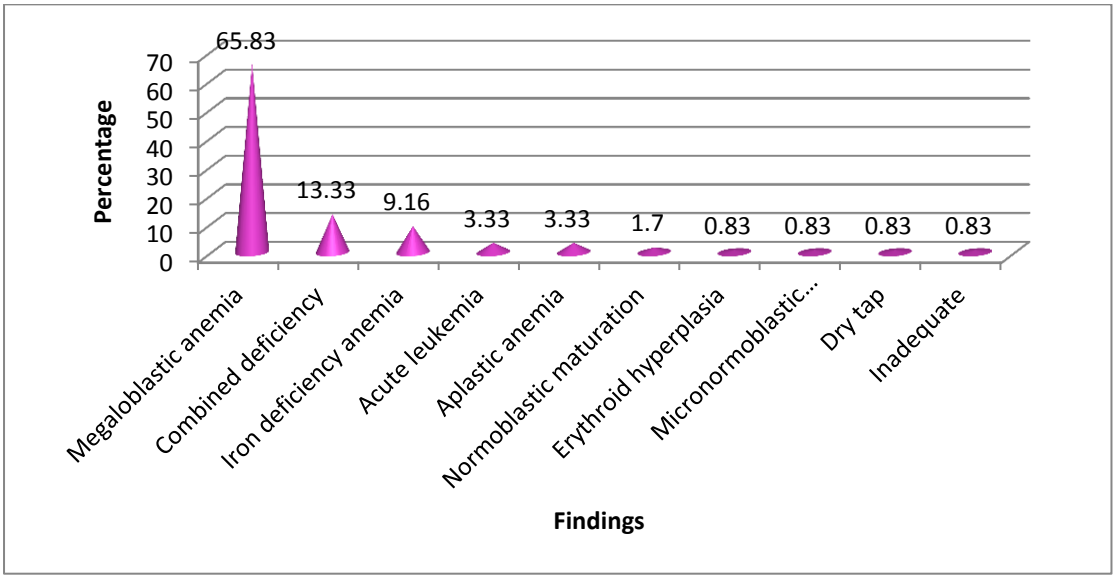
BONE MARROW ASPIRATION FINDINGS:

On bone marrow aspiration examination the predominant finding was megaloblastic anemia in 79 cases (65.83%) followed by combined deficiency anemia and iron deficiency anemia. Aplastic anemia and acute leukemia was diagnosed in 4 cases each.

TABLE 13: BONE MARROW ASPIRATION FINDINGS

Diagnosis	No. of cases	Percentage
Megaloblastic anemia	79	65.83
Combined deficiency	16	13.33
Iron deficiency anemia	12	9.16
Acute leukemia	4	3.33
Aplastic anemia	4	3.33
Normoblastic maturation	2	1.7
Erythroid hyperplasia	1	0.83
Dry tap	1	0.83
Inadequate	1	0.83
Total cases	**120**	**100**

FIGURE 10: BONE MARROW ASPIRATION FINDINGS



BONE MARROW BIOPSY FINDINGS:

Bone marrow biopsy was done in 23 cases. Biopsy findings were correlating with aspiration findings in 21 cases. In 16 cases megaloblastic anemia was seen, 2 cases of combined deficiency anemia and one each case of iron deficiency anemia and acute leukemia was observed. Bone marrow biopsy was useful in 2 cases, of which one had inadequate material and the other one was a dry tap, where diagnoses of myelodysplastic syndrome and megaloblastic anemia were made respectively.

TABLE 14: CORRELATION OF BONE MARROW BIOPSY AND ASPIRATION

Diagnosis	Bone marrow aspiration findings	Bone marrow biopsy findings correlating with aspiration	Bone marrow biopsy findings different from aspiration
Megaloblastic anemia	16	16	
Combined deficiency	2	2	
Iron deficiency	1	1	
Aplastic anemia	1	1	
Acute Leukemia	1	1	
Dry tap	1	-	1(MA)
Inadequate	1	-	1(MDS)
Total cases	**23**	21	2

DISTRIBUTION OF CAUSES OF PANCYTOPENIA:

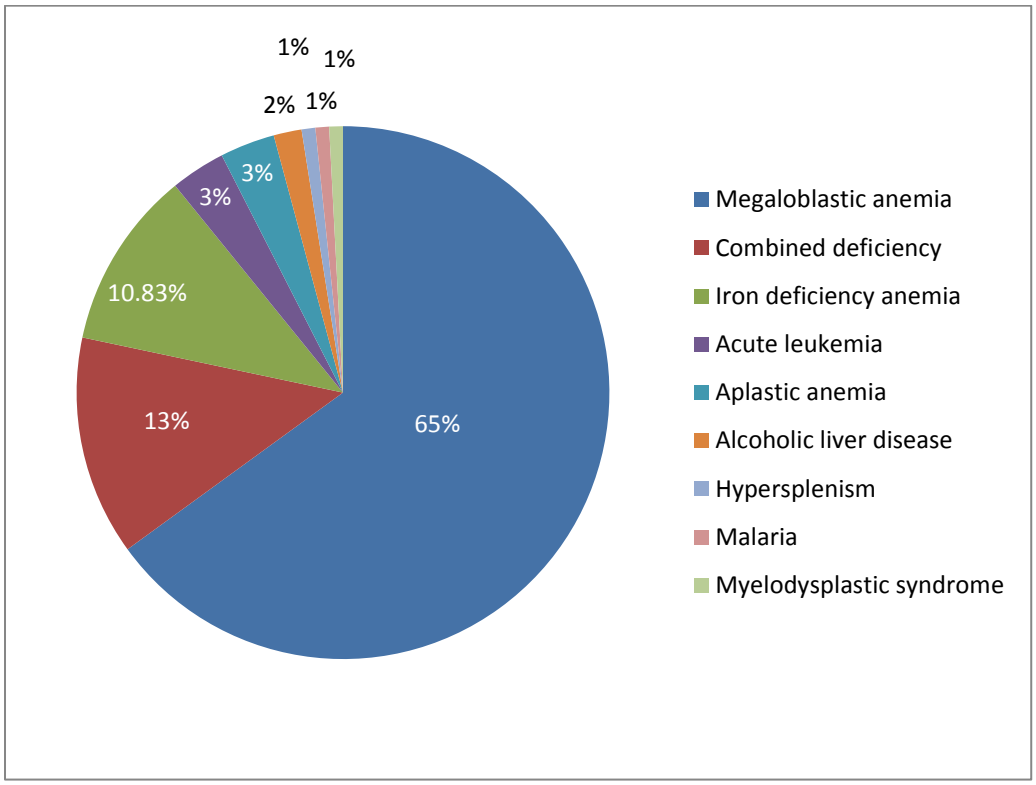
Distribution of causes of pancytopenia on peripheral smear examination, bone marrow aspiration and biopsy findings, clinical features and other relevant investigations were as follows:

In this study most common cause of pancytopenia is megaloblastic anaemia in 78cases (65%), second common cause is combined deficiency anaemia in 16 cases (13.33%), followed by iron deficiency anaemia (10.83%), acute leukemia and aplastic anaemia. Other causes are alcoholic liver disease, malaria, hypersplenism and myelodysplastic syndrome were made.

TABLE 15: DISTRIBUTION OF CAUSES OF PANCYTOPENIA

Diagnosis	**No. of cases**	**Percentage (%)**
Megaloblastic anemia (MA)	78	65
Combined deficiency anemia (CDA)	16	13.33
Iron deficiency anemia(IDA)	13	10.83
Acute Leukemia (AL)	4	3.33
Aplastic Anemia (AA)	4	3.33
Alcoholic Liver Disease (ALD)	2	1.7
Hypersplenism (HS)	1	0.83
Malaria	1	0.83
Myelodysplastic Syndrome (MDS)	1	0.83
Total	**120**	**100.00**

FIGURE 11: DISTRIBUTION OF CAUSES OF PANCYTOPENIA



HEMATOLOGICAL FINDINGS IN VARIOUS CAUSES OF PANCYTOPENIA

PANCYTOPENIA DUE TO MEGALOBLASTIC ANEMIA:

In the present study, megaloblastic anemia was found to be the commonest cause of pancytopenia and was seen in 65% of cases. Of these, it was observed in 51 (65.38%) males and 27 (34.61%) females (Table 17), with maximum number of cases seen in the 3rd, 4th and 5th decades of life (Table 16).

Predominant presenting complaint was generalized weakness (38 cases) followed by fever (17 cases).

History of alcoholism was present in 32 male patients, was found to be statistically significant ($p < 0.05$).

Pallor was present in all cases, 21 cases showed icterus and hepatosplenomegaly was present in 8 cases.

Maximum numbers of cases were having a hemoglobin level in the range of 3.5- 6gm%, WBC count between 2000- 3500 cells/cmm and platelet count between 4000- 50000 cells/ cmm.

On peripheral smear examination predominant RBC morphology was macrocytic in 59 cases, followed by dimorphic picture (with macrocytic and microcytic hypochromic cells) in 16 cases. Peripheral smear also showed hypersegmented neutrophils in 30 cases, nucleated RBCs in 14 cases, immature WBCs in 12 cases. (Fig. 5)

On bone marrow aspiration, cellularity was hypercellular with megaloblastic erythroid hyperplasia in majority of cases; 69 cases (88.5%). Megaloblasts are larger than normoblasts, nucleus showing characteristic feature of open sieve like chromatin. (Fig.6). Megaloblastic anemia cases also showed giant metamyelocyte and band forms with increased mitotic activity. (Fig. 7)

Bone marrow biopsy was done in 17 cases of megaloblastic anemia and showed hypercellularity with erythroid hyperplasia and predominance of immature erythroid precursors having large, round to oval nuclei with one to two nucleoli with moderate basophilic cytoplasm. (Fig.8)

PANCYTOPENIA DUE TO COMBINED DEFICIENCY ANEMIA

Combined deficiency anemia was the second most common cause of pancytopenia in present study seen in 13.33% of cases with equal distribution among both the sexes (Table 17). Majority of cases were between the age group of 16- 30 years of age (Table 16). Commonest mode of presentation was generalized weakness in 8 cases followed by giddiness. Pallor was present in all cases, 2 of cases showed icterus.

Hemoglobin ranged between 1.7 gm% to 8.5gm%, WBC count between 1230-3980 cells/cmm and platelet count ranged between 6000 to 139000 cells/ cmm.

On peripheral smear examination predominant RBC morphology was dimorphic with macrocytic and MCHC cells. (Fig. 9)

Bone marrow aspiration showed hypercellular marrow in all cases with erythroid hyperplasia with both megaloblastic and micronormoblastic maturation. (Fig. 10)

Bone marrow biopsy was done in 2 cases and correlated well with aspiration findings. (Figure 11 and 12)

PANCYTOPENIA ASSOCIATED WITH IRON DEFICIENCY ANEMIA:

13 cases (10.83%) of iron deficiency anemia were noted with predominant age group being 16- 30 (Table 18) years and with male preponderance (Table 17). Common presenting symptoms were generalized weakness and fever. Pallor was seen in all the cases and splenomegaly was present in 3 (27.3 %) of cases.

Hemoglobin value ranged from 3.3gm% to 9.3gm%, WBC count from 1780 – 3700 cells/cmm. Platelet counts ranged from 4000- 121000 cells /cmm.

RBCs on peripheral smear examination were microcytic hypochromic with moderate anisopoikilocytosis. (Fig. 13)

Bone marrow examination showed a hypercellular marrow with erythroid hyperplasia with micronormoblastic erythroblasts; the cells being smaller than normoblasts with ragged scant cytoplasm. Myelopoiesis and megakaryopoiesis were normal and showed sequential maturation. (Fig. 14 and 15)

Iron store on Perls' stain ranged from Grade - 0 to Grade 1 which is significantly lower than the normal store.

PANCYTOPENIA DUE TO ACUTE LEUKEMIA.

In the present study acute leukemia was present in 4 cases (3.33%) of which 2 were unclassified and 1 each were classified as APML and AML M2.

Age of these cases ranged from 2 years to 60 years. 3 cases were seen in less than 20 years of age, only 1 case was diagnosed in a 60 years old male, with female preponderance.

2 cases were presented with fever, and 1 had breathlessness and the remaining 1 case presented with bleeding tendency. On physical examination, 1 case had hepatosplenomegaly, lymphadenopathy and sternal tenderness.

Peripheral smear of all cases showed pancytopenia with blasts (Fig 18).

In a case of APML peripheral smear showed hypergranular promyelocytes with cytoplasmic granules and Auer rods. These cells showed MPO positivity ((Fig 16). Bone marrow aspiration showed 45% of promyelocytes (Fig.17).

Bone marrow aspiration showed > 20% of blasts in all the cases (Fig. 19).

Bone marrow biopsy was done in one case, showed hypercellular marrow with diffuse infiltration with blasts. These cells showed round to oval nuclei with prominent nucleoli and scant cytoplasm and there was marked suppression of erythroid series. (Fig. 20 and 21)

PANCYTOPENIA SECONDARY TO APLASTIC ANEMIA

In the present study, aplastic anemia was present in 4 cases (3.33%). Age of presentation ranged from 30 years to 65 years with female preponderance, with M: F ratio of 1: 3. Fever was predominant presenting complaint in 3 cases of Aplastic anemia, while 1 presented with generalized weakness. Hemoglobin value ranged from 1.7 to 6.3 gm%, total WBC count was between 970- 2670 cells/cmm and thrombocytopenia ranging from 5000 to 73000 cells /cmm.

RBC morphology was macrocytic in 2 cases and NCNC (Fig. 22) in 2 cases. In all cases bone marrow aspiration was hypocellular with increased adipocytes and suppressed erythropoiesis and granulopoiesis with predominance of lymphocytes and plasma cells (Fig. 23). Iron stores in 2 cases were Grade 5 (Fig. 24). Bone marrow biopsy was done in one case, which showed similar findings of a hypocellular marrow with predominance of lymphocytes and plasma cells. (Fig. 25) Study included single case of Rickettsial infection showing positivity on Weil Felix test.

PANCYTOPENIA DUE TO ALCOHOLIC LIVER DISEASE:

In the present study, 2 cases of with H/O chronic alcohol consumption were evaluated. One was a 59 years old male, who presented with generalized weakness and jaundice. He was found to have Hb of 5.9 gm%, leucopenia and thrombocytopenia. His PS showed macrocytic RBCs and Bone marrow was hypercellular with erythroid hyperplasia with megaloblastic maturation. His ultrasound findings of abdomen were consistent with Cirrhosis of liver. In another individual of 30 years age, who presented with loose stools, pallor and hepatomegaly, pancytopenia was seen with an Hb of 6gm%. Bone marrow aspiration was

normocellular with normoblastic maturation. The diagnosis of ALD was confirmed on ultrasound. This established a relation between ALD and pancytopenia.

PANCYTOPENIA ASSOCIATED WITH HYPERSPLENISM:

In present study pancytopenia was seen in a single case of Hypersplenism in 60 year old female, who presented with generalized weakness. On examination pallor and moderate splenomegaly were present. Peripheral smear showed dimorphic picture with normocytic normochromic cells and microcytic hypochromic cells with leucopenia and thrombocytopenia. Bone marrow examination was normocellular with normoblastic erythroid hyperplasia. Myeloid and megakaryocytic series were normal.

PANCYTOPENIA DUE TO MALARIA:

A 3year old male child who presented with fever and icterus and diagnosed to have malaria was evaluated in our study. Peripheral smear showed macrocytic RBCs, thrombocytopenia and leucopenia along with ring forms of Plasmodium falciparum (Figure 26). Bone marrow examination showed megaloblastic maturation.

PANCYTOPENIA DUE TO MDS:

In the present study a case of MDS had pancytopenia. The patient was a 58 year old female who presented with generalized weakness. She had a history of transfusion dependency and was refractory to hematinics. Her peripheral smear examination showed normocytic normochromic cells with leucopenia and thrombocytopenia. Bone marrow aspiration had inadequate material. Bone marrow biopsy showed hypocellular marrow for her age with erythroid hyperplasia with megaloblastic maturation (Fig. 27). Erythroid series showed features of

dyserythropoiesis (Fig. 28). Reticulin stain showed increased reticulin fibrosis with Grade 2 Myelofibrosis (Fig. 29).

TABLE 16: DISTRIBUTION OF CAUSES PANCYTOPENIA AMONG DIFFERENT AGE GROUPS

Cause	**Age group**					**Total**
	1-15	16-30	31-45	46-60	>60	
MA	6	22	20	21	9	78
CDA	1	4	2	4	5	16
IDA	1	5	3	3	1	13
AL	1	2	-	-	1	4
AA	0	1	1	1	1	4
ALD	-	-	1	1	-	2
Hypersplenism	-	-	-	-	1	1
Malaria	1	-	-	-	-	1
MDS	-	-	-	-	1	1

TABLE 17: GENDER DISTRIBUTION OF CAUSES OF PANCYTOPENIA

Causes	**Male (%)**	**Female (%)**	**Total No. of cases**
MA	51(65.38)	27 (34.61)	78
CDA	8 (50)	8 (50)	16
IDA	7 (54)	6 (46)	13
AL	1 (25)	3 (75)	4
AA	1 (25)	3 (75)	4
ALD	2 (100)	-	2
Hypersplenism	-	1 (100)	1
Malaria	1(100)	-	1
MDS	-	1(100)	1

BONE MARROW ASPIRATION AND BIOPSY NEEDLES:

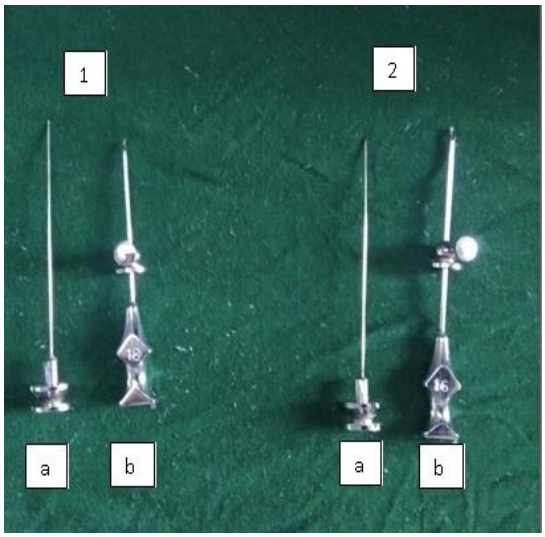


Fig 3: Salah Bone Marrow Aspiration needle. 1. Pediatric 2. Adult with stylet (a) and needle with guard (b).

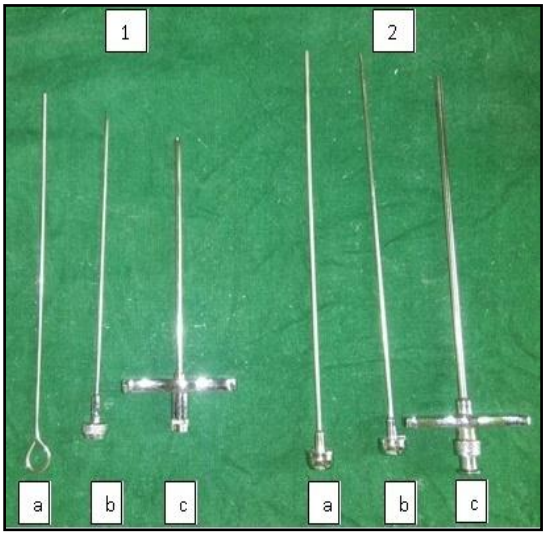


Fig 4: Jamshidi Bone Marrow biopsy needle, 1. Pediatric 2. Adult with needle (c), obturator (b), and stylet (a).

MEGALOBLASTIC ANEMIA:

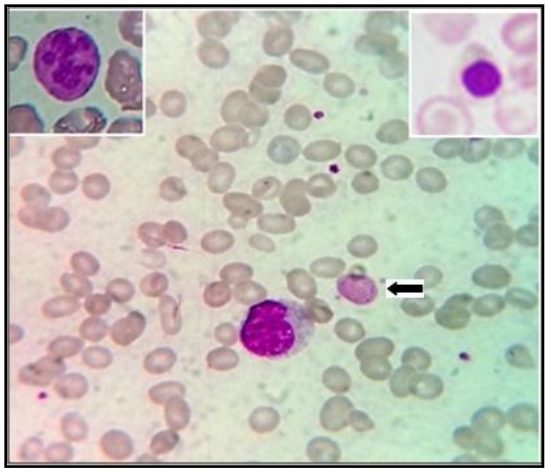


Fig 5: PS- Macrocytic anemia with Cabot ring (Arrow). Left Inlet shows hypersegmented neutrophils and right inlet shows nucleated RBC. (Leishman's stain, 1000x)

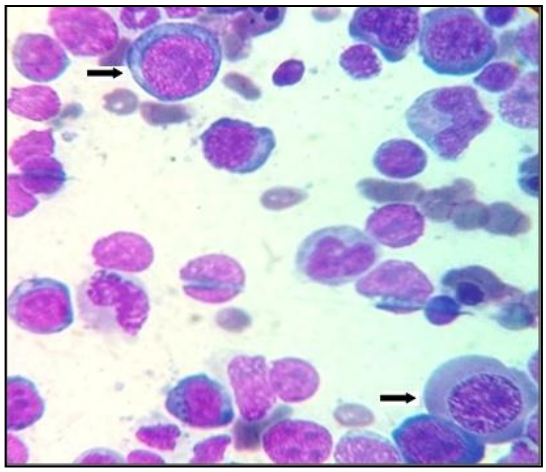


Fig 6: BMA – Megaloblasts with sieve like chromatin. (Arrow) (Leishman's stain, 1000X)

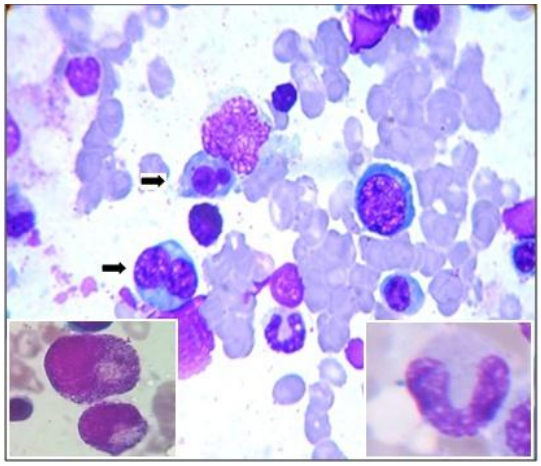


Fig 7: BMA- Dyserythropoiesis (Arrow), Left inlet shows Giant metamyelocyte, Right inlet shows Giant band form (Leishman's stain, 1000x)

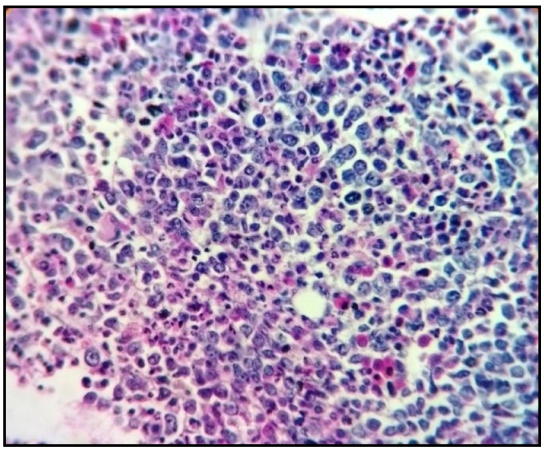


Fig 8 : BMB- Erythroid hyperplasia with immature erythroid precursors (H and E Stain 400x)

COMBINED DEFICIENCY ANEMIA:

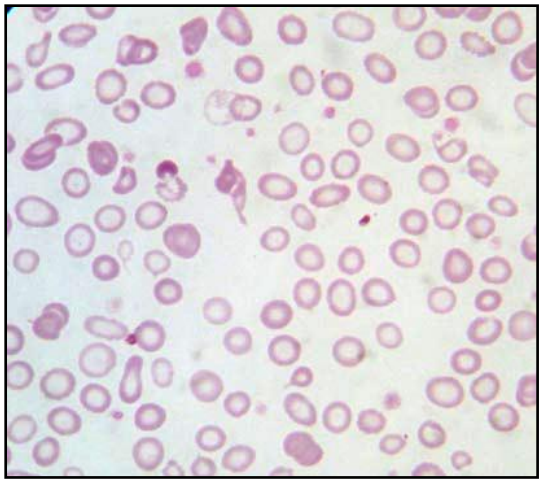


Fig 9 : PS- Both macrocytes and microcytic hypochromic RBCs (Leishman's stain, 1000x)

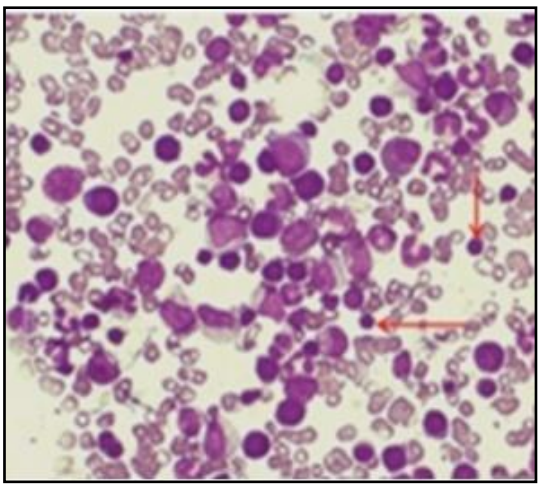


Fig 10 : BMA- Erythroid hyperplasia megaloblasts and micronormoblasts (Arrow) (Leishman's stain, 1000x)

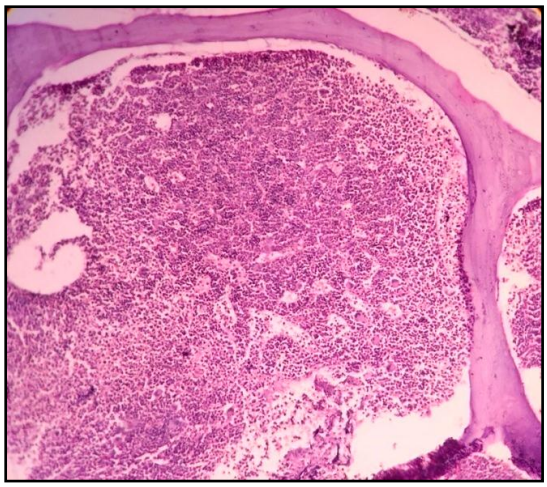


Fig 11: BMB- Hypercellular marrow (H and E Stain 400x)

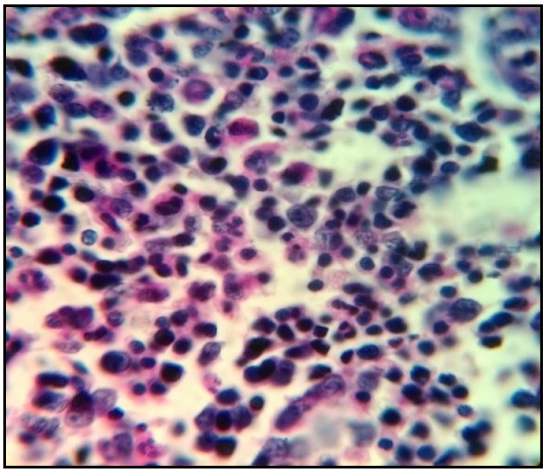


Fig 12: BMB- Megaloblasts and Micronormoblasts (H and E Stain 1000x)

IRON DEFICINCY ANEMIA

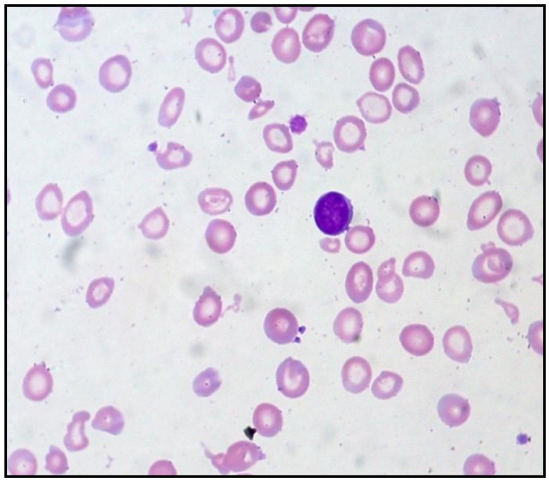


Fig 13: PS- Microcytic Hypochromic anemia (Leishman's stain, 1000x)

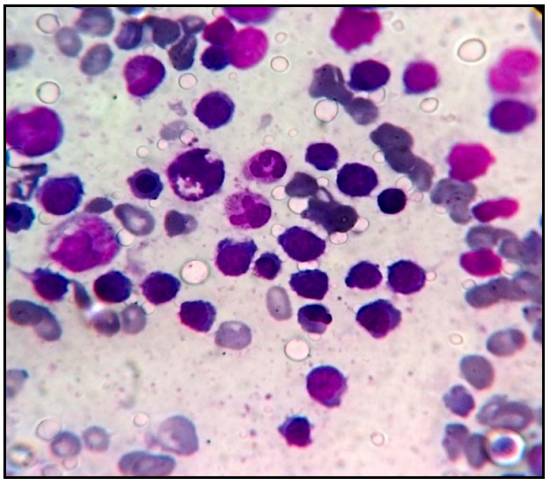


Fig 14: BMA- Micronormoblasts with poorly hemoglobinized ragged cytoplasm (Leishman's stain, 1000x)

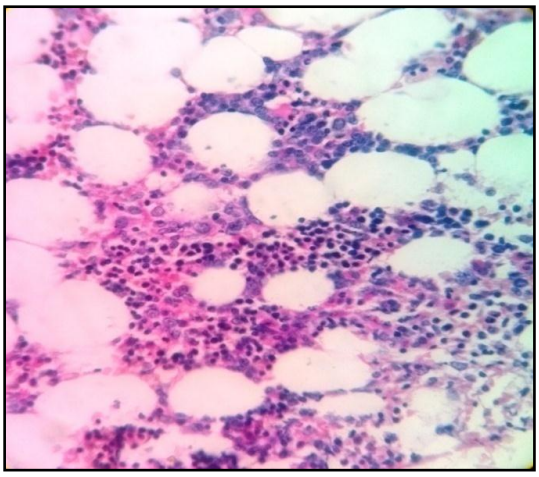


Fig 15: BMB- Erythroid hyperplasia with micronormoblastic maturation (H and E Stain 400x)

ACUTE LEUKEMIA:

ACUTE PROMYELOCYTIC LEUKEMIA:

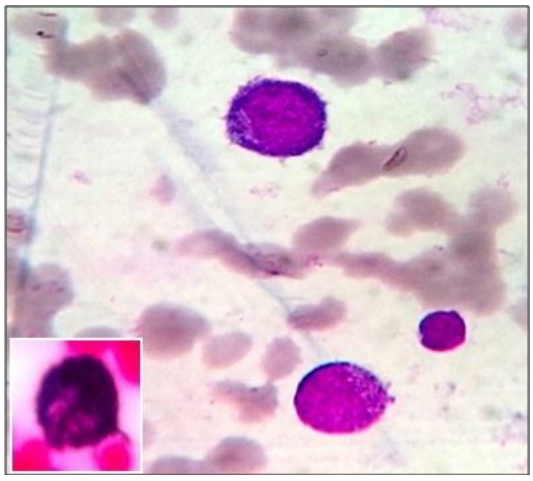


Fig 16: PS- Promyelocytes with cytoplasmic granules and Auer rods. Inlet shows MPO positivity. (Leishman's stain, 1000x)

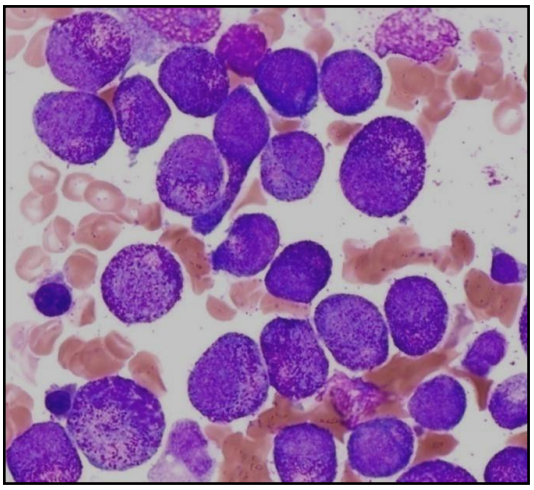


Fig17: BMA- Promyelocytes (Leishman's stain, 1000x)

ACUTE LEUKEMIA

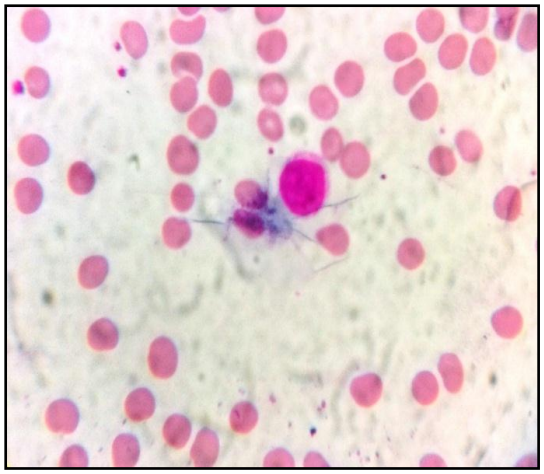


Fig 18: PS- Pancytopenia with blasts. (Leishman's stain, 1000x)

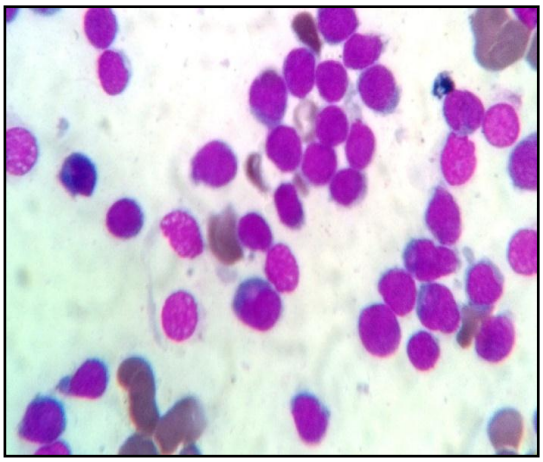


Fig 19: BMA- Showing > 20% of blasts. (Leishman's stain, 1000x)

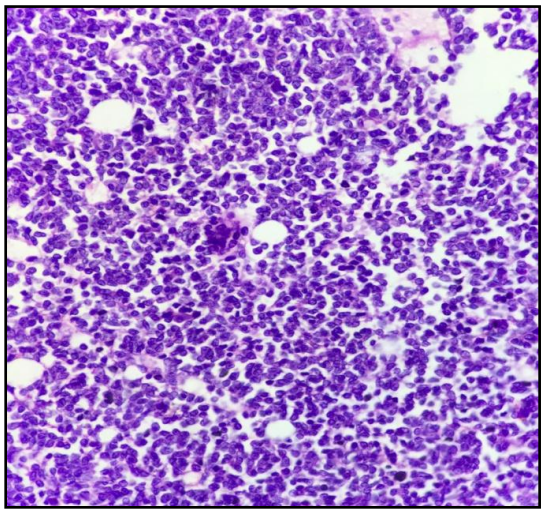


Fig 20: BMB— Sheets of blasts. (H and E Stain 400x)

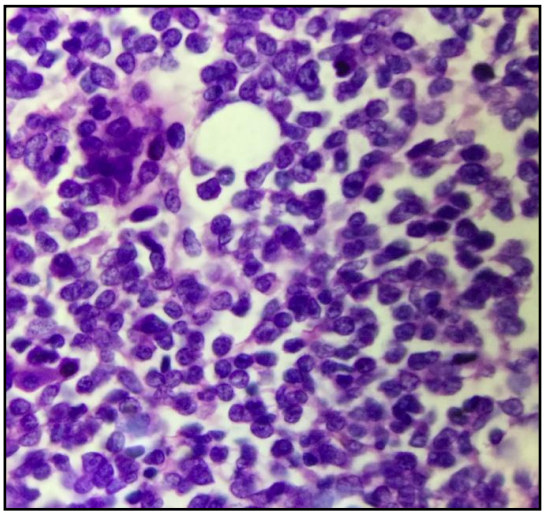


Fig 21: BMB— Sheets of blasts (H and E Stain 1000x)

APLASTIC ANEMIA:

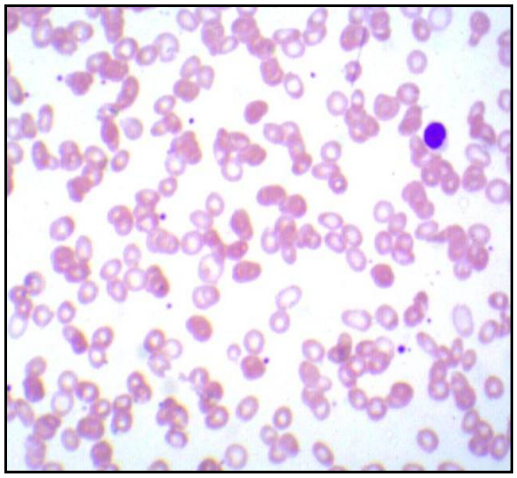


Fig 22: PS- Pancytopenia with NCNC RBCs (Leishman's stain, 1000x)

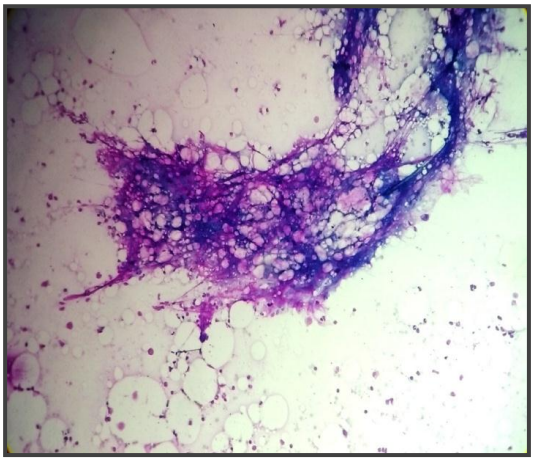


Fig 23: BMA- Hypocellular marrow with increased fat cells with predominant lymphocytes, plasma cells. (Leishman's stain, 1000x)

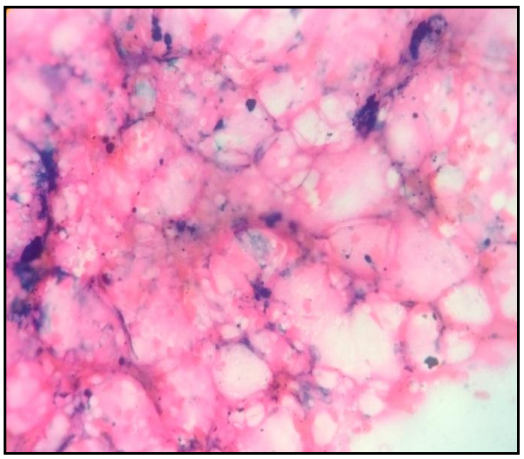


Fig24: BMA- Perls' stain show grade 5 iron stores.

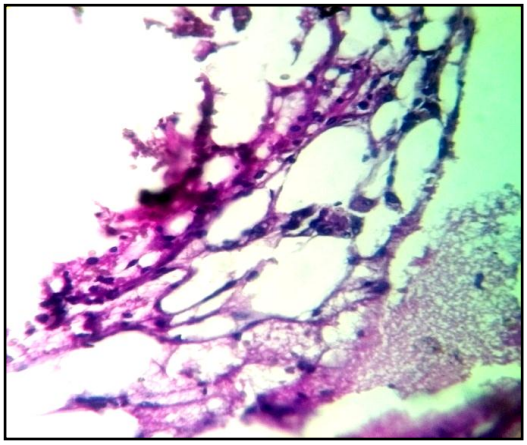


Fig 25: BMB- Hypocellular marrow with increased fat cells. (H and E Stain 400x)

MALARIA:

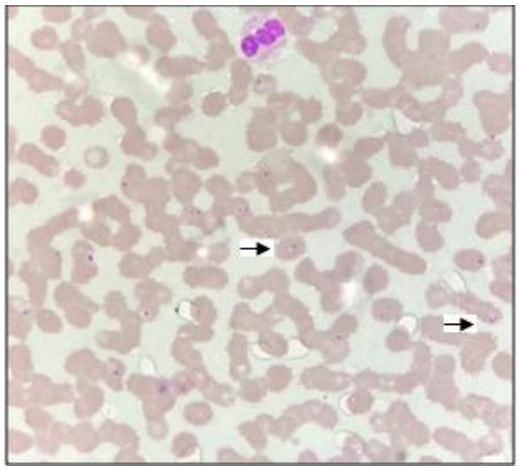


Fig 26: PS- Ring forms of Plasmodium falciparum (Arrow) (Leishman's stain, 1000x)

MYELODYSPLASTIC SYNDROME

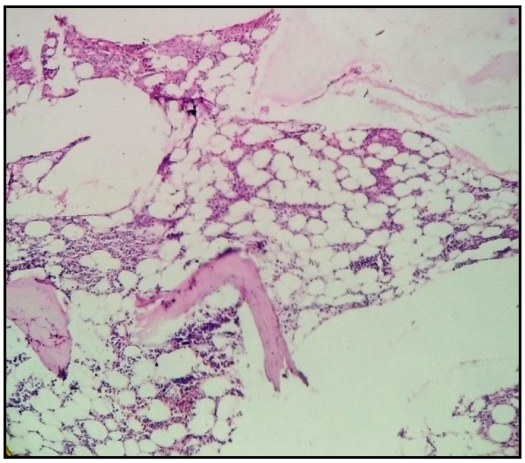


Fig 27: BMB- Hypocellular marrow (H and E Stain 400x)

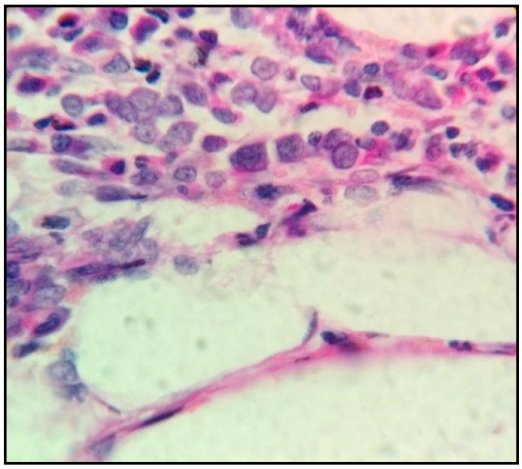


Fig 28: BMB- Erythroid hyperplasia with megaloblastic maturation showing features of dyserythropoiesis. (H and E Stain 400x)

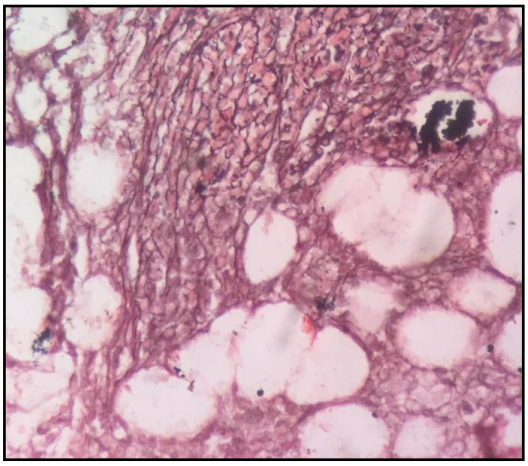


Fig 29: Reticulin stain shows Grade 2 myelofibrosis.

Pancytopenia is a common hematological finding with diverse clinical presentation. Due to wide spectrum of underlying etiologies, it often poses a diagnostic challenge. Therefore, a sound knowledge of accurate etiologies of this condition is crucial in the management of patients. [3] The variation in the frequency of diseases causing pancytopenia in different groups of population has been attributed to difference in methodology, diagnostic criteria, duration of follow up, geographic area, age distribution, nutritional status and prevalence of infective disorders. [8]

We studied a total of 120 cases of pancytopenia. Age and gender wise incidence, presenting symptoms were evaluated. Peripheral smear examination and bone marrow study was done in all cases. The observations and results were compared with previous published studies in literature.

TABLE 18: AGE DISTRIBUTION IN COMPARISON WITH OTHER STUDIES

Sl. No.	**Authors**	**Common Age Group Affected (years)**	**Number of cases**
1.	Javalgi AP *et al* [2](2013)	15-25	106
2.	Pasam R *et al* [10] (2016)	21-30	28
3.	Tejeswini V *et al* [8](2015)	51-60	75
4.	Devi PM *et al* [7] (2008)	21-40	50
5.	Khunger *et al* [3] (2002)	21-30	200
6.	**Present study**	**21-30 and 51- 60**	**120**

Our study correlates well with common age group was affected in studies done by Javalgi AP *et al* [2], Pasam *et al* [10], Tejeswini V *et al* [8], Devi PM *et al* [7] and Khunger *et al* [3] and. In present study 2nd and 6th decade is most commonly affected.

TABLE 19: SEX DISTRIBUTION IN COMPARISON WITH OTHER STUDIES

Sl. NO.	AUTHORS	M:F RATIO
1	Gayatri BN. *et al* [1] (2011)	1.2:1
2	Pathak R *et al* [4](2012)	1:1.04
3	Santra G et al [6](2010)	1.47:1
4	Reddy GP *et al* [5] (2016)	1.2:1
5	Khunger et al [3] (2002)	1.2:1
6	**Present Study**	**1.66: 1**

In all the above studies males were found to be affected more than the females. Present study also shows male preponderance with male: female ratio 1.66:1.

TABLE 20: PRESENTING COMPLAINTS IN COMPARISON WITH OTHER STUDIES

	No. of cases	Generalized weakness	Fever	Breathlessness	Bleeding manifestation
Gayatri BN *et al*[1] (2011)	104	100%	38.46%	43.26%	3.84%
Javalgi AP *et al*[2] (2013)	106	86.79%	58.49%	45.28%	2.83%
Santra G *et al*[6] (2010)	111	45.04%	50.45%	32.43%	41.44%
Tejeswini V*et al*[8] (2015)	75	28%	30.66%	9.33%	8%
Present study	**120**	**46.7%**	**24.2%**	**9.17%**	**5.83%**

In the present study, common presenting symptoms were generalized weakness (46.7% cases) and fever (24.2% cases). Similar features were noted in studies done by Gayatri BN *et al*[1], Javalgi AP *et al* [2], Santra G *et al* [6] and Tejaswini *et al* [8]. (Table 24)

Physical findings included pallor (100%), icterus (24.16%), splenomegaly (15%), hepatomegaly (11.6%), and sternal tenderness. These findings correlated with study done by Javalgi AP [2] with pallor 83.96%, spleenomegaly (17.92%) and hepatomegaly (24.52%).

TABLE 21: COMPARISON OF PERIPHERAL SMEAR FINDINGS WITH OTHER STUDIES:

Diseases	Total no. of cases			Anisopoikilocytosis			Nucleated RBCs			Hypersegmented neutrophils			Immature WBCs			reticulocytosis		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
MA	78	77	144	72	68	140	14	-	18	30	38	-	12	20	18	2	5	-
AA	4	19	28	1	17	2	-	-	2	-	5	-	1	-	-	-	-	-
CDA	16	-	-	16	-	-	1	-	-	-	-	-	1	-	-	2	-	-
IDA	13	-	-	13	-	-	-	-	-	-	-	-	-	-	-	2	-	-
Hypersplenism	1	-	6	1	-	3	-	-	-	-	-	-	-	-	-	-	-	-
MDS	1	-	4	1	-	-	-	-	-	-	-	1	-	-	3	-	-	-
Subleukemic leukemia	4	4	10	-	1	1	1	1	4	-	-	-	4	2	10	-	-	-
Malaria	1	2	2	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-
ALD	2	-	-	2	-	-	1	-	-	1	-	-	1	-	-	-	-	-
Multiple myeloma	-	1	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
dTB	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A: Present Study

B: Gayatri BN *et al* [1]

C: Khunger JM *et al* [3].

Anisopoikilocytosis was commonly present in cases of megaloblastic anemia and other conditions like myelofibrosis, Kala Azar, malaria and multiple myeloma. Megaloblastic anemia also showed hypersegmented neutrophils and reticulocytosis few cases.[9]

Oval macrocytes, some degree of anisopoikilocytosis with striking abnormal morphological forms including tear drop cells, fragments, basophilic stippling, Howell Jolly bodies and nucleated RBCs. Hypersegmented neutrophils are usually present in megaloblastic anemia. Hypersegmented neutrophils are highly suggestive of megaloblastic erythropoiesis but not pathognomonic. [14]

Anisopoikilocytosis was the predominant finding in our study. Peripheral smear study of megaloblastic anemia cases showed macroovalocytes, hypersegmented neutrophils and 2 cases showed reticulocytosis, which was also observed by Gayatri BN *et al*[1] and Khunger *et al* [3.] This demonstrates the value of PS study in the differential diagnosis of pancytopenia.[6]

PS study of CDA and IDA cases showed moderate to marked anisopoikilocytosis, tear drop cells, pencil cells.

In the present study nucleated RBCs were seen in cases of megaloblastic anemia and leukemia, which correlated with the study of Khunger *et al* [3].

Immature WBCs were seen in cases of megaloblastic anemia and leukemia, which correlated well with both the comparative studies.

BONE MARROW FINDINGS:

Bone marrow was hypercellular in 85% of cases, normocellular in 9.17% of cases and hypocellular in 4.17% of cases. This observation is comparable with study done by Javalgi AP *et al* [2]. They observed that the marrow was hypercellular in 68.8% and hypocellular in 16.2%. Santra J *et al* [6], in their study found that the marrow was cellular in 54.05% cases and hypocellular in 45.95 % cases.

In the present study hypercellular marrow was present in megaloblastic anemia, which correlated well with Santra J *et al* [6]. Hypocellular marrow was seen aplastic anemia. This observation correlated with study of Khunger *et al* [3].

Bone marrow biopsy findings were similar to aspiration in 21 cases, bone marrow biopsy was useful in two cases, as one was dry tap on aspiration and one was inadequate. Therefore, bone marrow biopsy should be performed simultaneously along with aspiration. Study done Pathak R *et al* [4] also conveyed the importance of bone marrow biopsy along with aspiration as 12 cases were inconclusive on aspiration, biopsy helped to conclude definite diagnosis.

TABLE 22: COMPARISON OF VARIOUS CAUSES OF PANCYTOPENIA WITH OTHER STUDIES

Study	No. of patients	Commonest cause	Other common causes	Rare cause
Gayatri BN *et al* [1]	104	MA (74.04%)	AA (18.26%), Leukemia (3.85%)	Malaria, MM, Storage disorder.
Khunger JM *et al* [3]	200	MA (72%)	Aplastic anemia (14%), Leukemia (5%)	Malaria, KZ, NHL, MF, MM, dTB
Javalgi AP *et al* [2]	106	MA (72.6%)	IDA (12.4%), Malaria (3.7%)	Leukemia, SLE, AA, MM, MF, MDS, HS
Santra G *et al* [6]	111	AA (22.72%)	Hypersplenism, CLD, KZ, Mixed deficiency anemia (6.31%), MA. SLE, drugs	Malaria, dTB, NHL, CLL, Leukemia, MDS
Reddy GP *et al* [5]	42	MA (38.1%)	Aplastic anemia (26.2%)	AML, Malaria, Malignancy, ALD, MM, Tuberculosis
Tejaswini V *et al* [8]	75	MA (68%)	Aplastic Anemia (13.3%)	Leukemia, Myelofibrosis, ITP, MM
Present study	120	MA (65%)	CDA (13.33%), IDA (10.83%),	AA, AL, ALD, HS, malaria, MDS

Megaloblastic anemia is the most common cause of pancytopenia in present study with 65% cases, which correlates well with studies done by Gayatri BN *et al*[1], Khungar JM *et al*[3] Javalgi AP *et al* [2] and Tejaswini V *et al* [8]. In our study there was association of megaloblastic anemia with alcoholism; similar association was observed by study done by Devi PM *et al* [7].

Second most common cause in our study is combined deficiency anemia (in 13.33% of cases). Santra G *et al* [6] reported 6.31% cases of mixed nutritional deficiency. This was not observed in the studies done by Gayatri BN *et al* [1], Khunger JM [3] *et al*. This may be due to the higher prevalence of nutritional defeciency in this part of India.

Agarwal R *et al* [9] studied 70 cases of pancytopenia, who encountered 7.14% of cases of mixed deficiency anemia, which was comparable with our study with 13.33% cases manifesting as combined deficiency anemia.

The next common cause of pancytopenia in our study was iron deficiency anemia with 10.83% of cases, which correlates with studies done by Javalgi AP *et al* [2] and Devi PM *et al* [7] with 12.2% cases and 8% cases respectively as iron deficiency anemia.

Though iron deficiency is associated with a reactive thrombocytosis, increasing severity of iron deficiency leads to normalization and occasionally even decrease platelet counts. The exact mechanism of this is unclear but may be related to the alteration in the activity of iron dependant enzymes in thrombopoiesis and leucopoiesis. [63] An association between thrombocytopenia and severe iron deficiency anemia has been observed due its essential role in later stages of thrombopoiesis.[7]

In our study we encountered 3.33% cases of acute leukemia which was also reported in the studies done by Gayatri BN *et al* [1] and Khunger J *et al* [3].

Aplastic anemia was the commonest cause of pancytopenia in a study done by Santra G *et al* [6] and Pathak R *et al* [4]. It was second common cause in studies done by Gayatri BN *et al*[1]and Khunger J *et al* [3]. In present study aplastic anemia was seen in 3.33% cases, thus the prevalence of aplastic anemia varies. This may be due to the

fact that the above studies were conducted in hospitals where majority of the cases were referred by community physicians.

In our study pancytopenia was present in 2 cases of alcoholic liver disease with 1.7% which correlated with findings of study done by Reddy GP *et al.*[5] In the present study Hypersplenism was present in one case, which correlated with study done by Javalgi AP *et al.* [2] Malaria was present in 1 case which correlated with the study done by Khunger J *et al* [3] .

Alcoholic Liver Disease, Hypersplenism, Malaria and MDS were found to be the rare causes of Pancytopenia.

CONCLUSION

Pancytopenia is an important clinico haematological problem encountered in clinical practice and should be suspected on clinical grounds when a patient presents with unexplained anaemia, prolonged fever and tendency to bleed.

The physical findings and peripheral blood picture provides valuable information in the work up of pancytopenic patients.

Evaluation of peripheral blood film reveals the most probable cause of anaemia, presence of nucleated RBC's and/or immature WBCs.

Bone marrow aspiration along with special stains is an important diagnostic tool in hematology that helps to evaluate various cases of cytopenia and is sufficient to make a diagnosis in cases of nutritional anemias and leukemias.

Megaloblastic anemia, Combined deficiency anemia and Iron deficiency anemia were the common causes of pancytopenia, suggesting a high prevalence of nutritional deficiency in this part of India. Therefore it is recommended that necessary steps be taken to correct such nutritional deficiencies.

The other common causes were acute leukemia and aplastic anemia. However, uncommon and rare causes such as Malaria, Myelodysplastic syndrome, Multiple myeloma, Storage diseases should be kept in mind while planning investigation for complete work up of cytopenic patients.

Present study concludes that a detailed study of heamogram along with bone marrow aspiration and biopsy in pancytopenic patients is necessary for evaluation of hematological disorders to reach a definitive diagnosis. Diagnostic clues obtained from evaluation of heamogram and bone marrow study was useful in early diagnosis and helpful in planning further investigations and management of pancytopenic patients.

- A present study "Pancytopenia- A clinico-hematological analysis" was undertaken at Shri B.M. Patil Medical College, Vijayapura.
- Total hundred and twenty patients of pancytopenia of all age group were evaluated.
- A combined evaluation of presenting complains, physical findings, primary haematological investigations and bone marrow study were done in all patients.
- Commonest age group affected was 3rd and 5th decade.
- Males accounted for 75 cases (62.5%) and female 45 cases (37.5%) with a M:F ratio of 1.66:1
- Commonest presenting complaint was generalized weakness followed by fever.
- Commonest physical finding was pallor followed by spleenomegaly and hepatomegaly.
- Megaloblastic anaemia (65%) was the commonest cause of cytopenia, followed by combined deficiency anaemia (13.33%) and iron deficiency anemia (10.83%).
- The study included other causes of Pancytopenia include acute leukemia and aplastic anemia.
- Rare causes were alcoholic liver disease, hypersplenism, malaria, myelodysplastic syndrome
- Lowest hemoglobin percentage was 1.7 gm/dl and noted in a case of aplastic anaemia.

- Lowest total leucocyte count was 500 cells/cmm and noted in a case of megaloblastic anaemia.
- Lowest platelet count of 4,000cells/cmm was noted in a case of megaloblastic anemia and iron deficiency anemia.
- Predominant red blood cell morphology observed on peripheral smear was macrocytic.
- Bone marrow aspiration was hypercellular in 102 patients, most commonly associated with Megaloblastic anaemia, followed by Combined deficiency anemia and Iron deficiency anemia.

REFERENCES

1. Gayatri BN, Rao KS. Pancytopenia: A Clinico Hematological Study. Journal of Laboratory Physicians. 2011;3(1):15–20.
2. Javalgi AP, Dombale VD. Clinico – Hematological Analysis of Pancytopenia : A Bone Marrow Study. National Journal *of* Laboratory Medicine. 2013;2(4):12–7.
3. Khunger JM, Arulselvi S, Sharma U, Ranga S, Talib VH. Pancytopenia-A Clinico-haematological study of 200 cases. Indian J Pathol Microbiol. 2002; 45(3): 375-9.
4. Pathak R, Jha a, Sayami G. Evaluation of bone marrow in patients with pancytopenia. J Pathol Nepal. 2012;2:265–71.
5. Reddy GPK, Rao KVM. Clinical features and risk factors of pancytopenia : a study in a tertiary care hospital. Int J Adv Med. 2016;3(1):68–72.
6. Santra G, Das BK. A cross-sectional study of the clinical profile and aetiological spectrum of pancytopenia in a tertiary care centre. Singapore Med J. 2010;51(10):806–12.
7. Devi PM, Laishram RS, Sharma PS, Singh AM, Singh MK, Singh YM. Clinico-hematological Profile of Pancytopenia in Manipur, India. Kuwait Med J. 2008;40(3):221–24
8. Tejeswini V, Premalatha P, Renuka I V. Clinicohaematological profile of Pancytopenia- A South Indian tertiary hospital experience. Indian Journal of Pathology and Oncology. 2015;2(September):165–9.
9. Agarwal R, Bharat V, Gupta BK, Jain S, Bansal R, Choudhary A et al. Clinical and Hematological Profile of Pancytopenia. International Journal of Clinical Biochemistry and research. 2015;2(1):48–53.

10. Pasam R, Garlapati, Chaganti P, Panchakarla G. " A Clinico-Hematological Study of Pancytopenia ". IOSR Journalof Dental and Medical Sciences. 2016;15(5):71–6.
11. Kumar V, Abbas AK, Aster JC. Diseases of White Blood cells, Lymphnode, Spleen, and Thymus In: Robbins and Cotran Pathologic Basis of Disease. 9th ed. Philadelphia: Elsevier Saunders; 2015.p.579-81.
12. Pancytopenia; Aplastic Anemia In: Saxena R, Pati HP, Mahapatra M, firkin F, Chesterman C, Penington D et al editors. de Gruchy's Clinical Haematology in Medical Practice. 6th ed. Wiley; 2013.p.107- 19
13. Kristi J, Smock, Perkins SL. Examination of the Blood and Bone Marrow. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F et al, editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2014.p.1-17.
14. Bain BJ, Clark DM, Wilkins BS. The normal bone marrow In: Bone Marrow Pathology. 4th ed.Wiley- Blackwell; 2010.p1–51.
15. Bates I, Lewis M. Reference ranges and normal values. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors. Dacie and Lewis Practical Haematology. 11th ed. Philadelphia: Elsevier Churchill Livingstone; 2012.p.11–7.
16. Dave UP, Koury MJ. Structure of the marrow and Hematopietic Microenvironment In: Kaushansky K, Prchal JT, Press OW, Lichtman MA, Levi M, Burns LJ et al, editors. Williams Hematology. 9th ed. New York: Mc Graw Hill; 2016.p.513–21
17. Găman A, Găman G, Bold A. Acquired aplastic anemia: Correlation between etiology, pathophysiology, bone marrow histology and prognosis factors. Rom J Morphol Embryol. 2009;50(4):669–74.

18. Teo J, Dror Y. Acquired Aplastic Anemia. Pediatr Hematol. 2006;3:64–76.

19. Young NS. Pathophysiologic mechanisms in acquired aplastic anemia. Hematology Am Soc Hematol Educ Program. 2006;72–7.

20. Shimamura A, Alter BP. Inherited Aplastic Anemia Syndromes In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F et al, editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2009.p.954–8.

21. Voda D, Deaconu a. Fanconi Anemia – Rare Cause of Pancytopenia At Child. Bulletin of the *Transilvania* University of Brasov. 2012;5(1).

22. Solomon PJ, Margaret P, Rajendran R, Ramalingam R, Menezes G a, Shirley AS, et al. A case report and literature review of Fanconi Anemia (FA) diagnosed by genetic testing. Ital J Pediatr. 2015;41(1):38. Available from:http://www.ijponline.net/content/41/1/38

23. Saha S, Banerjee A, Ranjan R, Acharya M, Hasan A, Sarkar N. Dyskeratosis congenita: a rare congenital pancytopenia. Int J Med Sci Public Heal. 2015;4(4):577. Available from: http://www.scopemed.org/fulltextpdf.php?mno=173528

24. Al-qahtani FS. Clinical Medicine Insights : Pathology Congenital Amegakaryocytic Thrombocytopenia : A Brief Review of the Literature. Clinical Medicine Insights: Pathology 2010;3: 25–30.

25. Ballmaier M, Germeshausen M, Schulze H, Cherkaoui K, Lang S, Gaudig A, et al. C-Mpl Mutations Are the Cause of Congenital Amegakaryocytic Thrombocytopenia. Blood 2001;97(1):139–46.

26. Dror Y, Freedman MH. Shwachman-Diamond syndrome: An inherited preleukemic bone marrow failure disorder with aberrant hematopoietic

progenitors and faulty marrow microenvironment. Blood 1999;94(9):3048–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10556188

27. Burroughs MD, Woolfrey A, Shimamura A. Shwachman Diamond Syndrome – a review of the clinical presentation, molecular pathogenesis, diagnosis, and treatmentblic Access. Hematol Oncol Clin North Am. 2009;23:233–48.

28. Arber D a, Orazi A, Hasserjian R, Borowitz MJ, Beau MM Le, Bloomfield CD, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127(20):2391–406.

29. Shah NM, Prajapati SG, Adesara RP, Patel a P. An analysis of 30 cases of myelodysplastic syndrome. Indian J Pathol Microbiol 2009;52(2):206–9.

30. Nilsson L, Astrand-Grundström I, Arvidsson I, Jacobsson B, Hellström-Lindberg E, Hast R, et al. Isolation and characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes: evidence for involvement at the hematopoietic stem cell level. Blood 2000;96(6):2012–21.

31. Porta MG Della, Malcovati L. Myelodysplastic syndromes with bone marrow fibrosis. Haematologica. 2011;96(2):180–3.

32. Ustwani O Al, Ford L a, Sait SJ, Block AW, Barcos M, Vigil CE, et al. Myelodysplastic Syndromes and Autoimmune Diseases -Case Series and Review of Literature. Leuk Res. 2013;37(8):894–9.

33. Copley GB, Schnatter a. R, Armstrong TW, Irons RD, Chen M, Wang XQ, et al. Hospital-Based Case-Control Study of MDS Subtypes and Benzene Exposure in Shanghai. J Occup Environ Med 2017;59(4):349–55. Available from: http://insights.ovid.com/crossref?an=00043764-201704000- 00001

34. Zahr AA, Salama ME, Carreau N, Tremblay D, Verstovsek S, Mesa R, et al. Bone marrow fibrosis in myelofibrosis: Pathogenesis, prognosis and targeted strategies. Haematologica. 2016;101(6):660–71.

35. Cervantes F, Dupriez B, Pereira A, Passamonti F, Reilly JT, Morra E, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. Blood. 2009;113(13):2895–901.

36. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006;3(7):1140–51.

37. Papadantonakis N, Matsuura S, Ravid K. Megakaryocyte pathology and bone marrow fibrosis: The lysyl oxidase connection. Blood. 2012;120(9):1774–81.

38. Passamonti F, Cervantes F, Vannucchi a. M, Morra E, Rumi E, Pereira A, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). Blood. 2010;115(9):1703–8.

39. Gupta P, Charan V, Kumar H. PNH revisited: Clinical profile, laboratory diagnosis and follow-up. Indian J Pathol Microbiol. 2009;52(1):38.

40. Notaro R, Gargiulo L, Angioletti M De, Rondelli T, Sica M. Recent advances in pathogenesis of paroxysmal nocturnal hemoglobinuria. DCTH. 2015;(4):53–64.

41. Wilcox BL a, Ezzell JL, Bernshaw NJ, Parker CJ, Wilcox L a, Ezzell JL, et al. Molecular Basis of the Enhanced Susceptibility of the Erythrocytes of Paroxysmal Nocturnal Hemoglobinuria to Hemolysis in Acidified Serum. Blood.1997;78(3):820–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1713516

42. Meri S, Morgan BP, Davies a, Daniels RH, Olavesen MG, Waldmann H, et al. Human protectin (CD59), an 18,000-20,000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. Immunology 1990;71(1):1–9.

43. Maciejewski JP, Follmann D, Rivera CE, Brown KE, Simonis T, Young NS. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and PNH/aplastic anemia syndrome. Blood 2001;98:3513–19.

44. Matloub YH, Brunning RD, Arthur DC, Ramsay NK. Severe aplastic anemia preceding acute lymphoblastic leukemia. Cancer 1993;71(1):264–8.

45. Villarreal-Martínez L, Carlos J, Marisol Rodríguez-Martínez J-P, González-Llano O, Gómez-Almaguer D. Acute lymphoblastic leukemia of childhood presenting as aplastic anemia: report of two cases. Rev Bras Hematol Hemoter 2012;34(2):165–7.

46. Breatnach F, Chessells JM, Greaves MF. The Aplastic Presentation of Childhood Leukaemia: a Feature of Common-ALL. Br J Haematol. 1981;49(3):387–93.

47. Kulkarni KP, Marwaha RK. Acute lymphoblastic leukemia with pancytopenia at presentation: clinical correlates, prognostic impact, and association with survival. J Pediatr Hematol Oncol 2013;35(7):573–6.

48. Emadi A, Baer MR. Acute Myeloid Leukemia In Adults. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F et al, editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2014.p.1577-9.

49. Kumar H, Buch A, Iqbal B, Kakrani A. Hypoplastic acute myeloid leukemia-M4: A rare case report. Med J Dr DY Patil Univ 2016;9(1):129-31.

50. Bennett JM, Orazi A. Diagnostic criteria to distinguish hypocellular acute myeloid leukemia from hypocellular myelodysplastic syndromes and aplastic anemia: Recommendations for a standardized approach. Haematologica. 2009;94(2):264–8.

51. Jain D, Singh T, Kumar N. Hypocellular acute myeloid leukemia with bone marrow necrosis in young patients: Two case reports. J Med Case Rep. 2009;3:1–4.

52. Sridevi HB, Rai S, Suresh PK, Somesh MS, Minal J. Pancytopenia in Multiple Myeloma- An Enigma: Our Experience from Tertiary Care Hospital. J Clin Diagn Res. 2015;9(11):EC04–6.

53. Means RT, Glader B. Anemia: General Consideration. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F et al, editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2014.p.595-7

54. Hoffbrand AV. Megaloblastic anemia. In: Hoffbrand AV, Higgs DR, Keeling DM, Mehta AB editors. Postgraduate Haematology. 7th ed. Wiley Blackwell; 2016.p.56-7

55. Aziz T, Ali L, Ansari T, Liaquat H Bin, Shah N, Ara J. Pancytopenia: Megaloblastic anemia is still the commonest cause. Pakistan J Med Sci 2010;26(1):132–6.

56. Varma N, Naseem S, Das R, Ahluwalia J, Sachdeva MS, Marwaha R. Pediatric patients with bicytopenia/pancytopenia: Review of etiologies and clinico-hematological profile at a tertiary center. Indian J Pathol Microbiol. 2011;54(1):75.

57. Barik S, Chandoke R, Verma A. A prospective clinico-hematological study in 100 cases of pancytopenia in capital city of India. J Appl Hematol. 2014;5(2):45. Available from: http://www.jahjournal.org/text.asp?2014/5/2/45/137139

58. Jhamb R, Kumar A. Iron deficiency anemia presenting as pancytopenia in an adolescent girl. Int J Adolesc Med Health. 2011;23(1):73–4.

59. Ballard HS. Alcohol-associated pancytopenia with hypocellular bone marrow. Am J Clin Pathol. 1980;73(6):830–4.

60. Manappallil RG. Acute onset pancytopenia following alcohol heavy drinking. Asian Journal of Bio-Medical Research. 2016;6–7.

61. Weston CF, Hall MJ. Pancytopenia and folate deficiency in alcoholics. Postgrad Med. 1987;63:117–20.

62. Jose W, Unnikrishnan A, Muthu P, Kumar K, Pavithran K. Aplastic anaemia complicating systemic lupus erythematosus (SLE) at presentation: A clinical vignette and review of literature. J Clin Diagnostic Res. 2011;5(3):637–9.

63. Voulgarelis M, Giannouli S, Tasidou A, Anagostou D, Ziakas PD, Tzioufas AG Bone marrow histological findings in systemic lupus erythematous with hematologic abnormalities: A clinicopathologcal study. AJH. 2006;81(8):590-7 Available from: http://www.ncbi.nlm.nih.gov/pubmed/17039179

64. Quiquandon I, Morel P, Lai JL, Bauters F, dresch C, Gluckman E et al. Primary Sjögren's syndrome and aplastic anaemia. Annals of Rheumatic Diseases. 1997;56:438–41

65. Hébert KJ, Huber SA, Willis K, Monier PL, Lopez FA. A Young Woman with Fever and Pancytopenia. J La State Med Soc. 2003; 155:192–7.

66. Gill MK, Makkar M, Bhat S, Kaur T, Jain K, Dhir G. Thrombocytopenia in malaria and its correlation with different types of malaria. Annals of Tropical Medicine and Public Health. 2013; 6(2):197-200

67. Vinoth PN, Thomas KA, Selvan SM, Suman DFR, Scott JX. Hemophagocytic syndrome associated with Plasmodium falciparum infection. Indian J Pathol Microbiol. 2011;54(3):594-6.

68. El-koumi MA, Afify M, Al-zahrani SH. A Prospective Study of Brucellosis in Children : Relative Frequency of Pancytopenia. Iran J Pediatr. 2014;24(2):155–60.

69. Deep HS, Kohli JS. Miliary Tuberculosis Presenting as Pancytopenia. Journal of Evolution of Medical and Dental Sciences. 2013;2(44):8465–7.

70. Avasthi R, Mohanty D, Chaudhary SC, Mishra K. Disseminated Tuberculosis : Interesting Hematological Observations. Case Reports. 2010;58243–4.

71. Yadav TP, Mishra S, Gupta VK, Siddhu KK, Bakshi G, Yadav RB. Pancytopenia in Disseminated Tuberculosis. Indian Pediatrics.1996;33:597–9.

72. Alghamdi AA, Awan FS, Maniyar IH, Alghamdi NA. Unusual Manifestation of Extrapulmonary Tuberculosis. Case Reports in Medicine. 2013;2013:5–8.

73. Opie J. Haematological complications of HIV infection. S Afr Med J. 2012;102(6):465–8.

74. Dikshit B, Wanchu A, Sachdeva RK, Sharma A, Das R. Profile of hematological abnormalities of Indian HIV infected individuals. BMC Blood Disorders. 2009;6:6–11.

75. Zota V, Braza J, Pantanowitz L, Dezube BJ, Pihan G. Solving Clinical Problems In Blood Diseases A 57-year-old HIV-positive man with persistent fever , weight loss , and pancytopenia. Am. J. Hematol. 2009;(84):443–6.

76. Weitzman S. Approach to Hemophagocytic Syndromes. Hematology Am Soc Hematol Educ Program. 2011;2011:178-83.

77. Moss P. The spleen. In: Hoffbrand AV, Higgs DR, Keeling DM, Mehta AB editors. Postgraduate Haematology. 7th ed. Wiley Blackwell; 2016.p.56-7

78. McGovern MM, Desnick RJ. Lysosomal abnormalities of the Monocyte-Macrophage System : Gaucher and Niemann-Pick Diseases. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F et al, editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins; 2014.p.1-17.

79. Zimran A, Elstein D. Gaucher Disease andRelated Lysosomal Storage Diseases. In: Kaushansky K, Prchal JT, Press OW, Lichtman MA, Levi M, Burns LJ et al, editors. Williams Hematology. 9th ed. New York: Mc Graw Hill;2016.p.1128-30

80. Pancytopenia; Aplastic Anemia In: Saxena R, Pati HP, Mahapatra M, firkin F, Chesterman C, Penington D et al editors. de Gruchy's Clinical Haematology in Medical Practice 6th ed.Wiley; 2013.p.332-35

81. Knupp C, Pekala PH, Cornelius P. Extensive Bone Marrow Necrosis in Patients With Cancer and Tumor Necrosis Factor Activity in Plasma. American Journal of Hematology. 1988;221:215–21.

82. Attili VS, Batra U, Loknath D, Dadhich HK, Madhumati M, Anupama G. A Case of Carcinoma Lung Presenting as Pancytopenia. Austral - Asian Journal of Cancer. 2006;5(3):131–2.

83. Spleen Hyperslenism and Splenomegaly In: Saxena R, Pati HP, Mahapatra M, firkin F, Chesterman C, Penington D et al editors. de Gruchy's Clinical Haematology in Medical Practice 6th ed.Wiley; 2013.p.332-35

84. Ishtiaq O, Baqai HZ, Anwer F, Hussain N. Patterns of pancytopenia patients in a general medical ward and a proposed diagnostic approach. J Ayub Med Coll Abbottabad . 2004;16(1):8-13.

ANNEXURE – I

INSTITUTIONAL ETHICAL COMMITTEE CLEARANCE CERTIFICATE

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103
INSTITUTIONAL ETHICAL COMMITTEE No/5 8/2015
20/11/15

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 17-11-2015 at 03 pm scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.

Title "Pancytopenia – A Clinico Hematological Analysis"

Name of P.G. Student : Dr Varsha Deshpande
Dept of pathology

Name of Guide/Co-investigator : Dr. R.M. Patekar, Professor

DR.TEJASWINI VALLABHA
CHAIRMAN
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization
1)Copy of Synopsis/Research Project
2)Copy of informed consent form.
3)Any other relevant documents.

ANNEXURE II

B.L.D.E.U's SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTER, VIJAYAPUR - 586103

RESEARCH INFORMED CONSENT FORM

I, the undersigned,_____ , S/O D/O W/O _____, aged ____years, ordinarily resident of _____ do hereby state/declare that Dr _____ of _____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases . Further the Doctor has informed me that he/she is conducting dissertation/research titled _____under the guidance of Dr _____requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

The Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further the Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept

secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment a follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of

treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

ANNEXURE-III

PROFORMA FOR STUDY

NAME	:	OP/IP No.	:
AGE	:		
SEX	:	D.O.A	:
RELIGION	:	D.O.D	:
OCCUPATION	:		
RESIDENCE	:		

Presenting Complaints :

Past history : History of drug intake,

Personal history :

Family history :

Treatment history :

General physical examination:

Pallor	present/absent
Icterus	present/absent
Clubbing	present/absent
Lymphedenopathy	present/absent
Edema	present/absent
Sternal tenderness	present/absent
Built	poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

Haematological investigations:

Parameters	
WBC	
RBC	
HGB	
HCT	
MCV	
MCH	
MCHC	
PLATELETS	
LYMPHOCYTES(%)	
NEUTROPHILS(%)	
EOSINOPHILS(%)	
MONOCYTES(%)	
BASOPHILS(%)	
RDW	
PDW	
MPV	
P-LCR	

Peripheral Smear Examination:

RBC:

WBC:

PLATELETS:

IMPRESSION:

Bone Marrow Aspiration Study:

Preparation	
Cellularity	
Erythroid series	
Myeloid series	
M:E Ratio	
Megakaryocytes	
Plasma cells	
Abnormal cells	
Parasites	
Pearl's stain	
IMPRESSION	

Specific investigations:

DIAGNOSIS:

ANNEXURE-IV

KEY TO MASTER CHART

Sl. No.	Serial No.
M	Male
F	Female
P	Present
A	Absent
Hb	Hemoglobin
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
Plt	Platelet
TC	Total count
PS	Peripheral Smear
AP	Anisopoikilocytosis
nRBCs	Nucleated Red Blood Cells
HSN	Hypersegmented Neutrophils
i.WBCs	Immature White Blood Cells
RC	Reticulocyte Count
Hyper	Hypercellular
Hypo	Hypocellular
Normo	Normocellular
N	Normal

↑	Increased
↓	Decreased
NS	NOT Seen
MA	Megaloblastic Anemia
CDA	Combined Deficiency Anemia
IDA	Iron Deficiency Anemia
AL	Acute Leukemia
AA	Aplastic Anemia
ALD	Alcoholic Liver Disease
HS	Hypersplenism
MDS	Myelodysplastic Syndrome